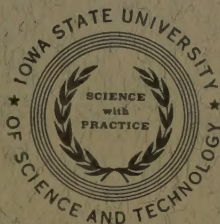


# IOWA STATE JOURNAL OF SCIENCE

*A Quarterly of Research*



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ABUNDANCE, GROWTH AND FOOD OF YOUNG GAME FISH  
IN CLEAR LAKE, IOWA, 1949 TO 1957<sup>1</sup>

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Abstract

Data on abundance and growth of young fish have been collected at Clear Lake each summer since 1949 by bag seining littoral areas at weekly intervals. Young yellow perch (Perca flavescens), largemouth bass (Micropterus salmoides), bluegill (Lepomis macrochirus) pumpkinseed (L. gibbosus), black crappie (Pomoxis nigromaculatus), and black bullheads (Ictalurus melas) were usually found in heavily vegetated habitats; walleye (Stizostedion vitreum) and white bass (Roccus chrysops) in moderately vegetated habitats, and yellow bass (R. mississippiensis) in sparsely vegetated habitats. Fewer data were collected on northern pike (Esox lucius), channel catfish (Ictalurus punctatus), yellow bullhead (I. nebulosus), smallmouth bass (Micropterus dolomieu) and white crappie (Pomoxis annularis).

Year class success for most species was good in 1951, 1954, and 1956 while poor in 1949 and 1950. Growth by most species was good in 1952, 1953, and 1955 but poor in 1950. Available data indicated no relationship between environmental conditions and year class success or growth except that growth was usually better when aquatic vegetation was more abundant. The growth of young yellow perch, largemouth bass, bluegills and pumpkinseeds, yellow bass, and black bullheads was apparently slower when these species were more abundant suggesting intraspecific competition while the growth of young largemouth bass, black crappies, and black bullheads was apparently affected by interspecific competition. Food was considered to be the primary limiting factor although other factors and particularly space limitations may have been involved. All species fed primarily upon Entomostraca early in the summer but immature insects, Hyaella, and fish became increasingly important as the fish grew larger.

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## Introduction

The first summer of life is one of the most critical periods of time during the life of fishes. If reproduction, survival, and development are good during the first few months after spawning, a given year class should usually produce good yields to the fishery. However, the factors affecting year class success and growth of fishes are often unknown or poorly understood.

The abundance and growth of young of the year fishes in Clear Lake, Iowa, have been estimated each summer since 1949 by Iowa Cooperative Research Unit biologists. These estimates were made by seining several areas of the littoral zone a number of times each summer. Some data were also collected in 1941, 1942, 1943, 1947, and 1948. In 1956 the author initiated a more systematic sampling program which involved two 75-foot seine hauls at each of 12 stations around the lake each week.

Clear Lake, Iowa is a 3,640 acre eutrophic lake with a maximum depth of 20 feet. The littoral zone, which is of primary importance in this study, is wide, often extending over 500 feet from the shore line. The bottom type of this zone is predominantly sand or fine gravel, with a thin organic deposit in the weed beds. Giant bulrush, Scirpus validus, and some pond weeds, Potamogeton spp. and Chara spp., are fairly abundant in several areas.

## Year Class Abundance

The estimates of abundance must be somewhat subjective since the amount of effort was not consistent prior to 1956, but the year classes can probably be satisfactorily rated as to good, fair or poor (Table 1). These classifications consider only relative abundance since 1949. It is probable that the yellow perch success even during the "good" years was not as large as prior to 1940 when the yellow bass began to increase in abundance (Carlander, Lewis, Ruhr and Cleary, 1953). The success of year classes of walleyes can probably be explained as the result of experimental stocking of walleye fry in the even-numbered years but not in the odd-numbered years. Except in 1949 and in 1955 when fair numbers of young walleyes were seined, few or no representatives of the unstocked year classes, 1949, 1951, 1953, 1955, and 1957, have been collected. Apparently stocking has increased the numbers of walleyes available to seining in Clear Lake. The year class success of the largemouth bass was aided in 1956 by stocking but other variations cannot be explained at this time. Yellow bass reproduction has been fairly successful in recent years but white bass were found in low numbers and were completely absent from the 1950 through 1953 collections. Since 1949 the bluegills and pumpkinseeds have not suffered a complete failure and it seems likely that the year class success of these species may often be determined after the first summer of life. Black crappies have shown somewhat less variation than some other species. Black bullheads seem to undergo periods of good reproduction and periods of poor reproduction. From 1948 through 1950 and from 1955 through 1957 reproduction by black bullheads was low while from 1945 through 1947 and from 1951 through 1954 good reproduction was observed (in part Forney, 1955).

Table 1. Classification of year class success as good, fair, or poor on the basis of young-of-the-year surveys and field observations, Clear Lake, Iowa, 1949-1957.

Yellow perch			Walleyes			Largemouth bass			Yellow bass		
Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor
1951	1949	1950	1950	1949	1951	1956	1949	1952	1950	1949	1953
1943	1952		1954	1952	1953	1957	1950		1951	1952	
1955	1954		1956	1955	1957		1951		1954	1955	
1956	1957						1953		1956	1957	
							1954				
							1955				

---

Bluegills and Pumpkinseeds			Black crappies			Black bullheads			White bass		
Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor	Good	Fair	Poor
1951	1949		1951	1950	1949	1951	1950	1949	1949	1954	1950
1953	1950		1954	1952		1952	1955	1957		1955	1951
1954	1952		1956	1953		1953	1956			1956	1952
1956	1955			1955		1954					1953
1957				1957							1957

It may be seen that in 1951, 1954, and 1956 conditions were favorable for the reproduction and early survival of several species of fish. On the other hand, only a single species had "good" success in 1949 and in 1955. Even the "good" success of white bass in 1949 did not produce as many fish as to be considered better than "fair" if compared on the same basis as the yellow bass. In no year was there a complete failure of more than two of the eight species considered. Also, most species had about the same success during a given year in five of the nine years. The most notable relationship between species occurred for walleyes and yellow bass which had about the same success in seven of the nine years, despite the fact that the walleye success is believed to be related to fry stocking. Maloney and Johnson (1957) noted a direct correlation between year class success of walleyes and yellow perch in two Minnesota lakes, but no such relationship was evident in this study.

The reasons why reproductive success and survival differ in various years are not easily discovered. Doan (1942) tried to relate the abundance of year classes of Lake Erie fishes to various meteorological and limnological conditions, but found few significant correlations.

Water temperature has a significant effect on the time of spawning of several species and also controls, to a great extent, the rate of development, hatching, and early growth. There are not sufficient data on water temperatures at Clear Lake for analysis, but in a shallow lake such as Clear Lake water temperatures fluctuate rather closely with air temperatures. Monthly mean air temperatures (Table 2) taken at the Mason



Table 2. Average monthly air temperatures (degrees Fahrenheit) for Mason City Airport, Mason City, Iowa, 1949-1957\*.

Year	May	June	July	August	Average
1949	60.2	70.9	74.3	71.8	69.3
1950	55.8	65.6	67.4	65.4	63.6
1951	60.4	62.8	70.0	67.0	65.0
1952	56.8	71.2	71.4	67.2	66.6
1953	57.8	70.2	71.2	70.6	67.4
1954	52.2	69.6	73.5	69.2	66.1
1955	61.3	65.2	76.8	75.0	69.6
1956	58.6	72.5	68.1	70.0	67.3
1957	55.8	66.1	75.8	69.6	66.8
Average	57.7	68.2	72.1	69.5	66.9

\* From "Climatological data. Iowa Section" of the U.S. Weather Bureau (1949-1957).

City Airport, which is about two miles east of Clear Lake, did not appear to be correlated with the success of year classes of the eight species.

Another factor which might have a major effect upon the success and survival of young-of-the-year fishes in Clear Lake is the amount of aquatic vegetation which may serve as cover and as a supporting surface for food organisms. The vegetation has not been quantitatively studied but an effort has been made to rank the years as to relative abundance of vegetative cover (Table 3). The yellow perch show the best correlation between abundance of vegetation and success of year classes: in 1950, when vegetation abundance was low, yellow perch had very poor reproductive success; in 1954, 1956, and 1957, when vegetation was moderate, perch success was rated as fair one year and good two years; and in three of the four years when vegetation was rated as abundant, perch success was rated good. Parsons (1950) suggested a relationship between aquatic vegetation and yellow perch abundance, growth, and condition.

Table 3. Abundance of aquatic vegetation in Clear Lake, Iowa, for the years 1950 through 1957\*.

Low	Moderate	High
1950	1954	1951
	1956	1952
	1957	1953
		1955

\* Parsons (1950) reported that vegetation was abundant in 1945 and 1946 but declined over the next 3 years.

A very important factor affecting the abundance of young fishes may be the degree of predation by larger fishes. Bailey and Harrison (1945) felt that predation was the principal factor maintaining a healthy balance among the fish populations of Clear Lake. Carlander *et al.* (1953) attributed the elimination of the 1950 year class of yellow bass before they were a year old to the poor hatch of yellow perch causing walleye predation to be centered on the young yellow bass. The population of forage fish other than young game and pan fish as indicated by the bag seine catches was below 20% of the total small fish population. Such a low population of minnows and other such forage fish would require that young game fish serve as a principal source of forage for larger fish.

It was thought that decreases in numbers of fish captured through the season might give clues to the mortality rates of young-of-the-year fishes. However, the catch data (Table 4) indicate no consistent decreases which might be attributed to mortality. Undoubtedly large numbers of fish die during their first summer, but new hatches, changed habits, and sampling variation seem to mask the effects of mortalities in the 1956 and 1957 samples.

The fact that the two major environmental factors considered, air temperatures and vegetation, were not closely correlated with year class abundance does not indicate that there was no relationship. Most likely the subjectiveness of the classification of year class abundance and the lack of extremes of temperatures and quantities of vegetation caused differences to be less obvious. If more precise estimates of year class abundance, water temperature changes, and vegetative abundance were obtained, relationships might be detected.

The 1956 and 1957 sampling was designed to sample all seinable habitats and to sample each station equally. It is, therefore, possible to learn something about the habitat selection of the various species from the seining data.

Yellowperch were more abundant in areas of heavy vegetation, showed some preference for moderately vegetated habitats as well in 1957, and were least abundant in habitats characterized by sparse vegetation. Walleyes and white bass seemed to require moderate amounts of vegetation and were seldom captured in areas with sparse vegetation and sandy bottom. Largemouth bass were relatively restricted to the habitats characterized by heavy vegetation and were less abundant where vegetation was generally moderate or sparse. Bluegills and pumpkinseeds, black crappies, and black bullheads had approximately the same requirements as largemouth bass. Yellow bass seemed to require areas where vegetation was sparse and the bottom was sand or gravel and were seldom captured in heavily vegetated habitats. Because of the similarity of habitat selection, certain species tend to be associated together.

#### Food Habits of Young-of-the-Year Fishes

The food habits of seven species of young-of-the-year fishes were tabulated for three time periods in 1956: those fish caught from June 17 to July 14; those caught from July 15 to August 11; and those caught from August 12 to September 1 (Table 5). The general trends in food habits,

Table 4. Comparison of numbers of some young-of-the-year fishes captured by equal amounts of bag seining each week, Clear Lake, Iowa, 1956 (first line) and 1957 (second line).

Species	6/16- 6/23	6/23- 6/30	6/30- 7/7	7/7- 7/14	7/14- 7/21	7/21- 7/28	7/28- 8/4	8/4- 8/11	8/11- 8/18	8/18- 8/25	8/25- 9/1
Yellow perch	9 3	144 39	123 20	304 45	116 28	106 29	132 51	123 60	122 43	67 50	102 100
Walleye	16 0	16 1	1 2	21 1	11 0	6 0	14 0	22 0	15 0	4 2	6 0
Largemouth bass	25 0	53 0	201 1	298 4	237 40	274 107	148 646	103 218	117 218	76 115	89 144
Bluegill and pumpkinseed	- -	- -	0 11	69 23	133 29	2075 26	1621 693	1666 458	1916 1133	1823 2189	2282 2257
Yellow bass	- -	- -	1 0	1 28	1934 701	244 222	286 395	457 319	2891 215	899 115	2072 247
Black crappie	- -	- -	2 0	0 3	4 11	15 5	51 23	74 17	175 40	38 1	191 26
Black bullhead	- -	- -	18 0	1580 0	1629 22	977 148	435 137	462 179	493 260	243 346	356 292
White bass	- -	- -	- -	- -	2 0	36 4	24 8	115 2	92 3	16 7	25 17



as the summer progressed and as the fish grew larger, were very similar to those reported for North Twin Lake fishes by Kutkuhn (1955). Early in the summer young yellow perch showed a preference for Entomostraca but later consumed more immature insects and Hyaella. Parsons (1950) noted numerous caddis flies and few Entomostraca in the stomachs of age group 0 yellow perch from Clear Lake although the rest of his findings agreed quite well with this study. Young walleyes changed from a diet of Entomostraca to predominately fish as the season progressed. Young largemouth bass ate Entomostraca early in the season but fed more on immature insects later during the summer. Young bluegills and pumpkinseeds preferred the Entomostraca throughout the season but consumed progressively more immature insects and Hyaella as they grew larger. Young black crappies fed almost entirely upon Entomostraca and some Hyaella throughout the season. Entomostraca were the preferred food of young yellow and white bass throughout the season but increasing amounts of immature insects were taken as the fish grew larger. No black bullheads, the other species which has been considered important throughout this study, were captured at either Station 3 or Station 8 in 1956. Forney (1955) found that young bullheads preferred Entomostraca when they were smaller and ate more Hyaella and insects later in the season.

Yellow perch, largemouth bass, bluegills, pumpkinseed, and black crappie apparently feed more during the daytime than at night while the reverse is true of walleye and yellow bass (Table 6).

#### Growth of Young-of-the-Year Fishes

The growth may be most easily determined by measuring the lengths of the fish collected on various dates. In some of the previous years standard lengths (from tip of snout to the end of the hypural plate) were used and in some other years total lengths were measured to the nearest tenth of an inch. In 1956 and 1957 all measurements were of total lengths in millimeters and all the previous measurements were converted to this form of measurement for the calculations which follow. Conversion of standard to total length was by means of the most appropriate conversion factors for each species reported by Carlander (1953).

Growth for the various years was compared by a method suggested by Carlander et al. (1953) which consisted of fitting straight-line regressions to the data (Table 7). The growth curve may be somewhat curvilinear but only growth from July 15 through August 31 was used and the growth rate appears to be fairly constant over this period. Each day was numbered consecutively starting with July 15 as 1 and ending with August 31 as 48. Mean lengths for particular days or weeks were used rather than individual observations.

The sizes of the various species on July 14, as in Table 7, were compared with the average temperatures (Table 2) for May and for June for the years 1949 through 1957 by the regression technique. Both positive and negative regressions were obtained, 13 and 3 respectively, and only one correlation coefficient was statistically significant ( $r$  for yellow bass size on May temperatures = 0.68 where  $r_{.05} = 0.63$  with 8 degrees of

Table 5. Food habits of some young fishes from Clear Lake, Iowa,

Species	Yellow perch			Walleye			Largemouth bass		
Dates <sup>a</sup>	A	B	C	A	B	C	A	B	C
No. examined	11	51	32	2	13	4	23	37	17
No. empty	0	5	0	0	4	2	0	4	0
Average total length (mm)	42	57	67	36	66	82	29	44	61
Food items									
Insect									
Chironomidae	31	15	10	-	21	-	13	25	7
Ephemera	25	21	1	-	-	-	20	20	1
Zygoptera	-	5	6	-	-	-	-	6	45
Corixidae	-	-	-	-	-	-	2	2	6
Adult Diptera	-	-	-	-	-	-	-	-	9
Other <sup>b</sup>	t	3	4	-	-	-	2	3	13
Unidentified	-	-	-	-	-	-	-	9	-
Crustacea									
Hyalella	17	40	51	-	-	-	2	4	4
Entomostraca	27	5	9	100	6	-	55	12	-
Unidentified	-	-	2	-	9	-	-	7	-
Fish	-	-	-	-	60	100	3	10	15
Other <sup>c</sup>	-	-	-	-	-	-	-	-	-
Unidentified	-	11	17	-	4	-	3	2	-

<sup>a</sup>Period A - June 17 to July 14,  
 Period B - July 15 to August 11,  
 Period C - August 12 to September 1

<sup>b</sup>Includes Anisoptera, Trichoptera, Coleoptera, Gerridae, Orthoptera, and Ceratopogonidae.

<sup>c</sup>Includes Hydracarina and Lyngbea.

t = trace.

expressed as the percentage of total stomach content, 1956.

Bluegill and Pumpkinseed			Black crappie		Yellow bass		White bass	
A	B	C	B	C	B	C	B	C
10	65	60	14	32	34	41	22	12
0	11	3	1	1	4	5	3	2
15	22	31	35	49	47	59	63	86
-	22	31	16	4	13	16	15	33
4	-	2	-	2	-	-	3	-
-	-	t	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	13
-	t	2	-	-	t	-	-	6
t	1	-	7	-	-	1	1	14
-	5	22	28	7	22	6	16	3
96	64	39	40	77	65	72	31	31
-	-	-	7	7	-	-	5	-
-	-	-	-	-	-	-	5	-
-	8	4	-	-	-	1	-	-
-	-	-	2	3	-	4	24	-



Table 6. Percentages of young fish with empty stomachs collected during the day (6 a.m. - 6 p.m.) and night (6 p.m. - 6 a.m.) at Clear Lake, Iowa, 1956.

Species	Day		Night	
	Number examined	Percentage empty	Number examined	Percentage empty
Yellow perch	51	0	42	12
Walleye	7	57	11	18
Largemouth bass	31	0	46	9
Bluegill and Pumpkinseed	76	0	59	24
Black crappie	15	0	31	6
Yellow bass	31	29	44	0
White bass	2	0	32	16

freedom). Also the growth rates of the various species between July 15 and August 31, b in Table 5, were compared with the average temperatures for July, August, and July and August for the years 1949 through 1957 by the regression technique. Again, no relationship appeared to exist between growth rates and temperatures and 13 of the regressions were positive while 11 were negative. Martin (1952) detected no relationship between the growth rate of lake trout in Ontario lakes and temperature.

Taking the length on September 1 (Table 7) as an index, the various calendar years may be rated as to the growth of the young-of-the-year fishes. In general few species showed good growth in 1950, 1956, and 1957. Most species showed slow growth in 1950, the coldest summer of the period (Table 2). Growth and vegetation (Table 3) seemed to be related. Young bluegills and pumpkinseeds, black crappies, yellow bass, and black bullheads all tended to grow faster or slower in proportion to the amounts of vegetation present. Whether the correlation between growth rate and vegetation was due to the effect of the vegetation as cover and a substrate for food or some other factor affecting both growth and vegetation simultaneously is not known.

There is no indication that years in which year classes were abundant were years in which growth was good (Table 8). The only species with which there was any relationship between growth during the first summer and the abundance of the year class was the yellow bass. In the years when yellow bass were abundant growth was less than in years when yellow bass were less abundant. The above observation suggests intra-specific competition.

The growth data collected in 1956 and 1957 for some young-of-the-year fishes were used to compare the growth rates of the various fishes at various locations. The same general regression methods applied to compare growth between years was used except that the unit of observation was a weekly mean length estimate for particular stations instead of the weekly mean length estimate for all stations. First, the growth rates

Table 7. Regressions of growth from July 15 through August 31 for some young-of-the-year fishes from Clear Lake, Iowa.

Species	Year	Number of observations	a	b	Estimated length on September 1 <sup>c</sup>
Yellow perch	1943	2	49.9	0.512	75.0
	1947	6	45.1	0.658	77.3
	1948	3	46.5	0.402	66.2
	1949	3	56.7	0.326	72.7
	1951	3	51.2	0.771	89.0
	1952	5	60.5	0.445	82.3
	1953	7	52.1	0.393	71.4
	1954	7	48.1	0.470	71.1
	1955	6	52.6	0.387	71.6
	1956	7	49.4	0.497	73.8
	1957	7	52.4	0.506	77.2
	1947-1957	54	51.6	0.462	74.2
Walleye	1943	2	78.6	1.544	154.3
	1948	3	78.1	0.725	113.6
	1949	2	106.6	0.490	130.6
	1950	10	62.5	1.104	116.6
	1952	3	86.7	1.507	160.5
	1954	6	83.6	1.221	143.4
	1955	3	86.5	1.367	153.5
	1956	7	59.4	0.662	91.8
	1947-1957	35	70.9	1.121	125.8
Largemouth bass	1943	2	57.0	0.188	66.2
	1949	2	9.0	1.800	97.2
	1950	5	22.2	0.640	53.6
	1951	3	35.2	0.743	71.6
	1952	3	44.7	0.071	48.2
	1953	7	45.3	1.287	108.4
	1954	6	40.0	1.210	99.3
	1955	6	42.8	0.578	71.1
	1956	7	42.2	0.467	65.1
	1957	7	31.9	0.632	62.9
	1947-1957	46	38.2	0.788	76.8

a The estimated length of the fish in millimeters on July 14 or the a term in the regression formula,  $\hat{Y} = a + bX$ .

b The estimated growth of the fish in millimeters per day between July 14 and August 31 or the b term in the regression formula,  $\hat{Y} = a + bX$ .

c The estimated length of the fish in millimeters on September 1,  $\hat{Y} = a + b(49)$ .

Table 7. (continued)

Species	Year	Number of observations	a	b	Estimated length on September 1 <sup>c</sup>
Yellow bass	1942	4	42.4	0.508	67.3
	1947	8	33.5	0.541	60.0
	1948	3	43.6	0.530	69.6
	1949	3	41.4	0.655	73.5
	1950	6	25.9	0.593	55.0
	1951	4	33.9	0.661	66.3
	1952	3	28.7	0.725	64.2
	1953	5	34.5	0.888	78.0
	1954	6	31.5	0.753	68.4
	1955	6	43.7	0.581	72.2
	1956	7	39.6	0.494	63.8
	1957	7	34.5	0.724	70.0
	1947-1957	58	34.4	0.678	67.6
Black crappie	1943	2	30.0	0.869	72.6
	1947	5	27.7	0.728	63.4
	1949	2	49.9	0.329	66.0
	1950	7	14.7	0.272	28.0
	1951	4	31.3	0.584	59.9
	1952	4	33.7	0.927	79.1
	1953	7	35.6	0.654	67.6
	1954	7	25.9	0.667	58.6
	1955	4	36.6	0.361	54.3
	1956	7	23.1	0.689	56.9
	1957	7	28.4	0.770	66.1
	1947-1957	54	26.4	0.718	61.6
Bluegill and Pumpkinseed	1943	2	22.2	0.175	30.8
	1949	2	6.5	0.819	46.6
	1950	3	11.8	0.160	19.6
	1951	3	16.4	0.554	43.5
	1952	4	22.4	0.240	34.2
	1953	7	16.9	0.433	38.1
	1954	7	18.2	0.233	29.6
	1955	6	18.2	0.418	38.7
	1956	7	18.4	0.306	33.4
	1957	7	19.9	0.192	29.3
	1947-1957	46	18.6	0.294	33.0
Black bullhead	1943	2	30.2	1.144	86.3
	1951	3	33.0	0.161	40.9
	1952	5	35.7	0.880	78.8
	1953	7	28.8	0.605	58.4
	1954	7	31.9	0.495	56.2
	1955	6	32.9	0.619	63.2



Table 7. (continued)

Species	Year	Number of observations	a	b	Estimated length on September 1 <sup>c</sup>
	1956	7	33.1	0.428	54.1
	1957	7	25.4	0.626	56.1
	1947-1957	42	31.2	0.556	58.4
White bass	1949	3	77.6	-0.410	57.5
	1954	5	41.6	1.512	115.7
	1955	6	63.3	0.760	100.5
	1956	7	47.0	0.974	94.7
	1957	6	44.2	1.084	97.3
	1947-1957	28	53.2	0.898	97.2

Table 8. Computed total length in millimeters as of September 1 for young fish from Clear Lake, Iowa, for year classes rated as poor, fair, or good in abundance.

Species	Abundance		
	Poor	Fair	Good
Yellow perch	-	71, 73, 77, 82	71, 72, 74, 89
Walleye	108	131, 154, 160	92, 117, 144
Largemouth bass	48	54, 71, 72, 97, 99, 108	63, 65
Bluegill and Pumpkinseed	-	20, 34, 39, 47	29, 30, 33, 38, 44
Black crappie	66	28, 54, 66, 68, 79	57, 59, 60
Yellow bass	78	64, 70, 72, 74	55, 64, 66, 68
Black bullhead	56	54, 63	41, 56, 58, 79
White bass	97	95, 100, 116	58

of a given species were compared between the stations at which over 10% of the total catch of the species being considered was captured and the stations at which less than 2% of the total catch of the same species was captured for the period of July 15 to August 31 (Table 9). Such comparisons should indicate possible effects of intraspecific competition. Next, growth rate comparisons were made for each species between the three stations yielding the highest total catches of all species and the three stations of lowest total catches (Table 10). The latter comparisons should indicate possible effects of interspecific competition.

Yellow perch seem to have been their own most serious competitor. Even though yellow perch were fairly closely associated with largemouth bass, bluegills and pumpkinseeds, black crappies, and black bullheads as far as habitat requirements are concerned, their food habits were apparently different enough so that they only competed with themselves.

Since a small sample of walleyes was available only in 1956, no definite statement can be made concerning competition involving this species. It might be expected that no interspecific competition would affect walleyes at stations with high populations of other fish since their food habits change to fish quite early and few other highly predacious young fish are present.

The results of the regression analyses of largemouth bass growth were quite erratic. However, a trend towards both intraspecific and interspecific competition seems evident especially in 1956 when young-of-the-year fish populations were larger.

Bluegills and pumpkinseeds also possibly are affected by intraspecific and interspecific competition but a late hatch of these species in 1957 makes interpretation rather difficult.

Evidence of competition influencing the growth of black crappies was limited to possible interspecific competition. The dependence of this species on Entomostraca, which was also the main food of most other species early in the summer and to some extent all summer, might explain the observed effects on black crappies.

Yellow bass data consistently indicated effects of intraspecific competition. The increased effect of intraspecific competition within large schools was indicated by data collected in 1956 (Figure 1). Since yellow bass were not associated with any other species as far as habitat requirements were concerned, no interspecific competition was expected.

Black bullheads seemed to suffer both intraspecific and interspecific competition. Since this species tends to school and generally occupied the same habitats as yellow perch, largemouth bass, bluegills and pumpkinseeds, and black crappies, both types of competition might have actually affected the growth of black bullheads if food were the limiting factor.

Relatively low numbers of white bass were collected which reduced the possibility of intraspecific competition involving this species. Since white bass were generally not found where other species were abundant, little evidence of interspecific competition involving this species was expected or obtained.

Differences between growth rates at the various stations seem to have been related to large numbers of fish and common food habits. Therefore, food was probably the primary limiting factor. Competition might be strongest early in the summer when most species eat Entomostraca

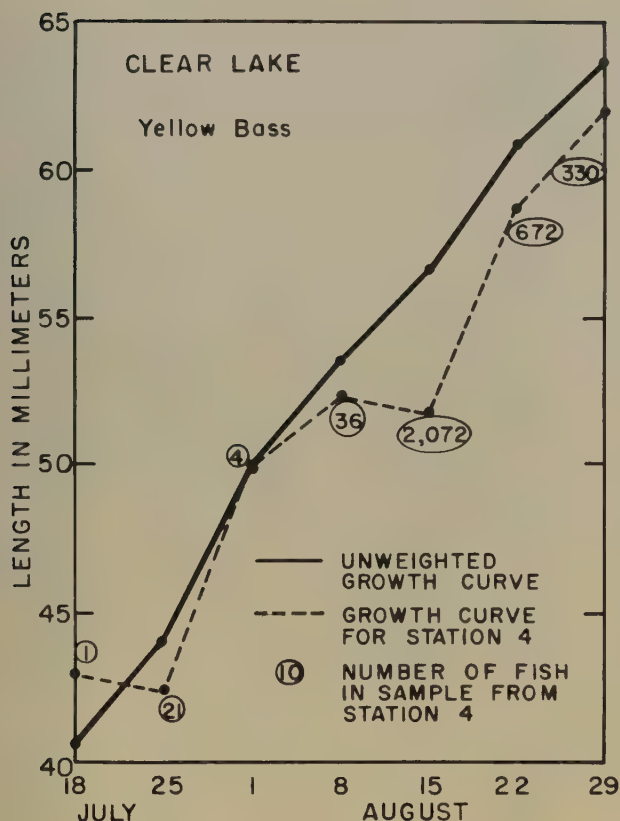


Fig. 1. Plotted growth curves for young-of-the-year yellow bass comparing the unweighted curve for all stations with the Station 4 curve to illustrate the relative size of fish in large schools with the over-all population, Clear Lake, Iowa, 1956.

but effects at that time could hardly be detected with the data and techniques used in the above analysis. Since the reduced growth rates noted for the various species could generally be explained by competition for food, it is believed that the effects of environmental changes such as low water levels were essentially small. Further support of this belief is the fact that water levels were even lower in 1957 than in 1956 but evidence of competition was not as great. However, population levels were also lower in 1957 which does not exclude the possible effect of space on the growth. There are no quantitative data with which to prove or disprove either hypothesis.



Table 9. Regressions of growth for some young-of-the-year fishes from Clear Lake, Iowa, calculated from the weekly mean lengths at stations yielding more than 10 per cent of the total catch of each species (top line) and stations yielding less than 2 per cent of the total catch of each species (bottom line), July 15 - August 31, 1956 and 1957.

Species	Year	Number of observations	a	b	Estimated length on September 1 <sup>c</sup>
Yellow perch	1956	12	51.5	0.37	69.6
		12	52.8	0.60	82.2
	1957	24	53.0	0.44	74.6
		6	49.5	0.66	81.8
Largemouth bass	1956	14	47.0	0.35	64.2
		14	34.8	0.60	64.2
	1957	20	32.2	0.67	65.0
		15	22.9	0.78	61.1
Bluegill and Pumpkinseed	1956	35	19.4	0.27	32.7
		18	15.7	0.31	30.9
	1957	31	20.6	0.15	28.0
		31	20.6	0.11	26.0
Black crappie	1956	17	23.8	0.65	55.6
		9	27.8	0.50	52.2
	1957	13	29.8	0.63	60.7
		4	28.9	1.08	81.8
Yellow bass	1956	13	38.1	0.52	63.6
		11	29.7	0.80	68.9
	1957	17	35.0	0.69	68.8
		4	37.1	0.77	74.8
Black bullhead	1956	11	33.9	0.34	50.6
		10	36.0	0.53	62.0
	1957	23	31.3	0.40	50.9
		4	2.9	1.34	68.6
White bass	1956	15	49.2	0.89	92.8
		4	52.9	0.76	90.1

<sup>a</sup>The estimated length of the fish in millimeters on July 14 or the a term in the regression formula,  $\hat{Y} = a + bX$ .

<sup>b</sup>The estimated growth rate of the fish in millimeters per day between July 15 and August 31 or the b term in the regression formula,  $\hat{Y} = a + bX$ .

<sup>c</sup>The estimated length of the fish in millimeters on September 1,  $\hat{Y} = a + b(49)$ .

Table 10. Regressions of growth for some young-of-the-year fishes from Clear Lake, Iowa, calculated from the weekly mean lengths at the three stations yielding the largest total catches of all species (top line) and the three stations yielding the lowest total catches (bottom line), July 15 - August 31, 1956 and 1957.

Species	Year	Number of observations	a	b	Estimated length on September 1 <sup>c</sup>
Yellow perch	1956	17	52.5	0.52	78.0
		14	55.0	0.52	80.5
	1957	12	50.6	0.62	81.0
		7	52.7	0.50	77.2
Walleye	1956	8	56.3	0.75	93.0
		11	64.1	0.43	85.2
Largemouth bass	1956	14	38.9	0.49	62.9
		11	33.0	0.62	63.4
	1957	20	34.2	0.70	68.5
Bluegill and Pumpkinseed	1956	21	19.4	0.23	30.6
		19	14.9	0.42	35.5
	1957	19	22.2	0.11	27.6
		16	19.8	0.13	26.2
Black crappie	1956	12	24.5	0.59	53.4
		10	25.0	0.65	56.8
Yellow bass	1956	13	37.9	0.57	65.8
		17	39.3	0.56	66.7
	1957	6	32.8	0.86	74.9
		13	35.3	0.68	68.6
Black bullhead	1956	11	33.9	0.34	50.6
		6	36.4	0.50	61.0
	1957	19	32.7	0.32	48.4
White bass	1956	7	47.4	0.96	94.4
		12	46.8	0.96	93.8
	1957	2	41.8	1.07	94.2
		4	45.9	1.05	97.4

<sup>a</sup>The estimated length of the fish in millimeters on July 14 or the a term in the regression formula,  $\hat{Y} = a + bX$ .

<sup>b</sup>The estimated growth rate of the fish in mm/day between July 15 and August 31 or the b term in the regression formula,  $\hat{Y} = a + bX$ .

<sup>c</sup>The estimated length of the fish in mm on September 1 =  $\hat{Y} = a + b(49)$ .

## SUMMARY BY SPECIES AND NOTES ON OTHER SPECIES

## Northern Pike

During recent years the numbers of northern pike in Clear Lake have been largely dependent upon stocking (Ridenhour, 1957). Growth by this species is generally good and young-of-the-year over 10 inches in total length have been captured by the first of August. The stomach content of only one young northern was examined during this study and it contained an unidentified fish. In 1956 the young northern, until they were about eight inches long, were restricted to the weedier areas where they had been stocked. Thereafter, they were captured in areas where cover was not particularly good.

## Channel Catfish

Bailey and Harrison (1945) doubted that channel catfish could reproduce in Clear Lake. However, in 1954 two young-of-the-year of this species were seined indicating at least some successful reproduction that year. Young-of-the-year channel catfish were fairly common in the bag seine catches of 1956 and 1957. Since most of the catfish captured have been seined at night, the lack of collections prior to 1954 might be explained by the very little night seining done before 1956. Whether natural reproduction can maintain the population of this species in Clear Lake remains to be seen. At present this species supports only a small portion of the total fishery (DiCostanzo and Ridenhour, 1957).

Most of the catfish captured have been in habitats characterized by moderate vegetation and sandy bottoms. The food of two specimens examined was Entomostraca, *Hyaella*, and immature insects. By September 1 this species averaged about  $2\frac{1}{2}$  inches in total length.

## Black Bullhead

Eyed eggs in black bullhead nests were observed as early as June 19, 1957, but the young did not appear in the bag seine catches until mid-July of that year. They were first captured during the first week of July in 1956. The young were generally concentrated in heavily vegetated areas where large schools could often be observed. In 1954, when a very large hatch of bullheads was observed, the schools could be found around most of the lake. No stomachs of this species were examined during this study but Forney (1955) indicated that they eat Entomostraca and immature insects.

Very large hatches of bullheads seem to be required to obtain successful year classes. Little indication of fish older than age group 0 has been found for the 1955 and 1956 year classes when reproduction was not particularly good. In fact the cyclic occurrence of good and poor year classes mentioned earlier suggests the characteristics of a "bound" lake (Eschmeyer, 1937). Bailey and Harrison (1945) felt that predation on this species was less than for some others but that it did occur.

## Yellow Bullhead

Yellow bullheads were considered "occasional" in Clear Lake by Bailey and Harrison (1945) but very few young were captured by the Unit biologists until 1956. It is believed that the main reason young of this species had not been collected in the past was because seining was generally restricted to daylight hours. During this study numerous young yellow bullheads were captured but usually at night. Just where they went during the day is not known except Bailey and Harrison (1945) indicated that they may hide under stones and other objects.

Generally yellow bullheads grew at the same rate as black bullheads. The stomachs of two individuals examined contained mostly immature insects and some Hyalella.

## White Bass

While the yellow bass have been increasing in numbers during recent years, the white bass have declined (Lewis, 1950). The controlling mechanisms of this decline are not known. During the past four years white bass have reproduced successfully which they had not done from 1950 through 1953. How the recent reproductive success compares with the reproductive success 20 years ago is not known but it is assumed that reproduction was much better earlier. Since this species does not seem to compete directly with yellow bass during their first year of life, there appears to be no relationship between the population changes which can be attributed to interspecific strife during the first summer of life. Relatively little is known about the spawning habits of the white bass. Bonn (1953) indicates that when running water, which is their preferred spawning habitat, is absent they will spawn on wind-swept points in Lake Texoma. Possibly the lack of suitable areas has caused the decline of white bass in Clear Lake since there are no significant inlets to the lake. However, lack of suitable spawning areas does not seem to explain their earlier success and recent decline. Possibly there was sufficient inflow of waters during years of normal rainfall, whereas this inflow has been lacking in recent drought years. Sigler (1949) noted fluctuations of the white bass populations of Spirit Lake, Iowa, and also considered the ability to reproduce a possible controlling factor. At Clear Lake increased predation upon the eggs or newly hatched fry by the recent large yellow bass populations may be a factor.

White bass did not appear in the seine catches until early or mid-July when they were approximately two inches long. There is no information available to indicate why this species was not collected earlier since Sigler (1949) felt that this species spawns during the last week of May in Spirit Lake, Iowa, which is quite similar to Clear Lake physically and geographically. During the summer the white bass seem to prefer the moderately vegetated areas. Their food was predominately Entomostraca early in the summer but many insects, immature and adult, and some fish were eaten later. By September 1 most of the young white bass are about  $3\frac{3}{4}$  inches long.



### Yellow Bass

Yellow bass apparently spawn during late May and early June around the Island and possibly near other rocky shores. Yellow bass were first captured by seining about the first week of July when the fish were a little over 1 inch long. As for white bass, the lack of young yellow bass collected before July cannot be explained.

This species seems to prefer the sparsely vegetated areas and sandy bottoms. Often they are found in large schools in their preferred habitat but schooling was less apparent in other areas. They feed predominately upon Entomostraca and some Hyalella and immature insects. Little competition seems to affect this species except from other young yellow bass. By September 1 they are usually about 3 inches in length.

Yellow bass have been of particular interest at Clear Lake in recent years because of large population variations (DiCostanzo and Ridenhour, 1957). Since its introduction some time prior to 1932 when they first appeared in the fishermen's catch (Bailey and Harrison, 1945), this species has become one of the most abundant species in Clear Lake. No real clues to the factor or factors controlling the population levels of this species were obtained during this study.

### Largemouth Bass

Largemouth bass first appeared in the bag seine catches in late June. They were mainly concentrated in areas of heavy vegetation. Their food was at first Entomostraca but they soon ate increasing quantities of insects and some fish. By the first of September they were nearly 3 inches long although this species exhibits large variation of growth rate and some individuals may be almost 6 inches long.

Even though large numbers of young largemouth bass have been stocked into Clear Lake in recent years and natural reproduction has seemed successful, the numbers of adult bass have not increased noticeably. Apparently the habitat needed by this species is not available in sufficient quantity to support a larger population.

### Smallmouth Bass

Formerly smallmouth bass were fairly common in Clear Lake (Bailey and Harrison, 1945) but since 1943 only two young-of-the-year have been collected. There seems to be no obvious explanation for the practical elimination of this species. Possibly environmental changes related to maturity of the lake, increased housing development around the lake, and increased boating activities have altered or reduced their required habitat.

### Bluegill

Bluegills first appeared in the bag seine catches about the first or second week of July. The first sign of reproduction by bluegills during 1957 was eyed eggs found in nests along the north shore in the bulrush areas on June 19. Apparently spawning by this species may continue

through the first of August. The young can be found around the entire lake but were concentrated in areas of heavy vegetation. Their food was principally Entomostraca until they were about  $1\frac{1}{2}$  inches long when increasing amounts of immature insects were eaten.

Even though good hatches have been common for this species during the last few years, good survival of the year class beyond age group 0 has been limited to occasional years (DiCostanzo, 1957). Since there are young of this species entering the population throughout the summer, they are probably very important as a first source of fish food for the young predators.

### Pumpkinseed

Pumpkinseeds seem to have about the same characteristics as bluegills. They are practically indistinguishable from bluegills when encountered in the field before they are somewhat over 1 inch in length. For this reason they have been lumped with the bluegills during this study. As an example of their relative abundance, an attempt was made to identify the pumpkinseeds collected during the last three weeks of August, 1956, and they were found to constitute about 4% of the total bluegill-pumpkinseed catch.

### Black Crappie

Black crappies were first caught with the bag seine during the first week of July. They were found principally in the heavily to moderately vegetated areas. Their food was almost entirely Entomostraca throughout the first summer of life. By September 1 they reached an average length of about  $2\frac{1}{2}$  inches.

### White Crappie

White crappies are less important to the Clear Lake fishery than black crappies. However, there was indication that the white crappies may become increasingly important. Generally, this species was not sampled with the 30-foot bag seine but there is reason to believe that the bag seine used during this study did not properly sample this species. Large numbers of young white crappies were observed by the writer when the Lake Survey crew of the Iowa State Conservation Commission sampled Clear Lake in the summers of 1956 and 1957 with a 500-foot bag seine. These results seem to indicate that white crappies require the deeper open waters not sampled by the smaller seine.

Other than habitat requirement differences, the habits of the white crappies appeared to be similar to those of the black crappies. The stomachs of 20 fish were examined and their food was almost entirely Entomostraca with a few immature insects included. Their growth was slightly faster than that of black crappies and they were about  $2\frac{3}{4}$  inches long by September 1.

## Yellow Perch

Young yellow perch first appeared in the bag seine catches about mid-June. They usually were found in heavily to moderately vegetated areas in association with young largemouth bass, bluegills and pumpkinseeds, black crappies, black bullheads, or walleyes. At first they fed on Entomostraca but later during the summer they ate large numbers of immature insects and *Hyalella*. By September 1 this species was usually about 3 inches long. The young yellow perch are important as forage for predator species and for walleyes in particular (Bailey and Harrison, 1945).

In recent years there has been a general decline of the perch population. Data collected during this study were inadequate to determine whether or not the reproductive success has declined since 1950. Reproduction has been fairly constant during the past four years but has been known to drop drastically during years when vegetation was sparse in Clear Lake (Carlander et al., 1953).

## Walleye

Young walleyes appeared in the bag seine catches about the same time as the yellow perch. A survey made during the 1956 spawning season indicated that walleyes spawn to some extent around most of the eastern end of the lake with the exception of the bulrush areas. Their principal spawning areas were around the Island, around Dodge's Point, and near the Outlet. Stocking of walleye fry has been conducted for a number of years and starting in 1949 when no stocking was done, stocking has been conducted every other year to determine the value of the program. Generally year class success has been best for those years when stocking was done. Predation on the eggs by other fishes and fungus killing the eggs are possible factors which were observed in studies on the efficiency of the natural reproduction in 1955 and 1956.

The young walleyes seemed to require the moderately vegetated areas. Their food habits changed from Entomostraca to fish when they were about 2 to 3 inches long. Growth has been generally good with the fish reaching a total length of about 6 inches by September 1 during most years.

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DRYING AND MATURITY OF GRAIN SORGHUM  
AS AFFECTED BY WATER LOSS FROM PLANT PARTS<sup>1</sup>

Ivan Wikner and R. E. Atkins<sup>2</sup>

Grain sorghums are a popular and productive crop in much of the Southern Great Plains. In areas of limited rainfall they generally are a more reliable crop than corn. In recent years a striking increase in production of grain sorghum has taken place in states to the north and east of the main area of production. The advent of high yielding hybrids, the occurrence of several abnormally dry seasons and the popularity of sorghums as a substitute feed crop for acres diverted from corn under acreage allotment programs all have contributed to the expanded grain sorghum acreage in this area.

Climatic conditions during some fall seasons in the corn belt area may be unfavorable for normal maturation and drying of sorghum grain. Frequent rains and the occurrence of many days during October and November of 1957 which were not favorable for drying grain resulted in many acres of grain sorghum that did not attain a grain moisture content suitable for harvest and storage without artificially drying the grain. In other recent years sorghum producers in this area have been able to harvest sorghum grain by mid- to late-October at moisture contents suitable for storage. Thus it appears that severity of the sorghum grain moisture problem may vary considerably from year to year.

Hybrids or varieties which mature early and dry rapidly undoubtedly will be favored for production under these conditions. The purpose of this investigation was to study the rate of water loss from the grain and other plant parts of several grain sorghum hybrids and varieties, and to determine if loss of water from the grain was associated with the rates of water loss from other plant parts.

PERTINENT LITERATURE

Published reports of studies with grain sorghum which relate directly to this investigation are limited in number. However, several studies with corn, which may be applicable to this study, have been reported. As corn and sorghum are similar morphologically and in many growth characteristics, certain of the studies with corn also will be reviewed.

Newlin (8) found that water loss from the grain, cobs and stalks of corn was relatively slow and followed an essentially linear pattern, while

<sup>1</sup>Journal Paper No. J-3794 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1364. Part of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree at Iowa State University of Science and Technology.

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water loss from the leaves, husks, and shanks was more rapid and was not linear in nature. Differences in rates of water loss between the two groups were attributed to basic morphological differences of the respective plant parts. Water loss from the shanks was rapid after a killing frost and there was an increased rate of water loss from the stalks after a freeze. His experiments indicated that the differential rate of water loss among the six single crosses studied was governed by genetic factors. He also observed that for some lines of corn the grain dried rapidly in comparison with the cobs, but the comparative rate of drying for grain and cobs was reversed with other strains. Rate of water loss from other plant parts did not have a measurable effect on rate of water loss from the grain.

Climatic effects on the rate of drying with corn were observed by Schmidt (9) to be greatest when kernel moisture was above 50% or below 25%. He concluded that when grain was within the 25 to 50% moisture range, the effects of climatic fluctuations were slight if the corn had been planted at normal planting time and plant growth and development was normal.

Physiologic maturity has been defined by Aldrich (2) and by Shaw and Loomis (11) as the point at which maximum dry weight of the grain is attained. Aldrich (2) stated that moisture in corn grain averaged 35% when translocation ceased, and that general plant appearance was not a reliable index of relative or actual maturity. Maximum dry weight of the grain was obtained at 29.2, 35.2, and 40.0% moisture for early, medium and late maturing corn hybrids, respectively, in experiments reported by Shaw (10).

Dessureaux *et al.* (4) found that some strains of corn reached 35 to 40% grain moisture before maximum dry weight was attained, while other strains reached this moisture level after maximum dry weight had been obtained. They also reported that the expression of heterosis in kernel development affected the rate of moisture depletion in grain, and that maturity was hastened 4 to 5 days with cross-pollination as opposed to self-pollination. They concluded that strains of corn that flower early tend to mature rapidly.

Aboul-Ela (1) found that maximum dry weight of corn grain was not always attained when moisture content of the grain reached 35 to 40% and that the point at which maximum dry weight was obtained varied considerably among hybrids and with seasons. Observations on the moisture content of corn grain at physiologic maturity also have been reported by Hallauer (5) and by Kiesselbach (7), and generally were within the ranges presented by other investigators. Maximum dry weight of sorghum grain was obtained approximately 30 days after bloom in studies reported by Clegg *et al.* (3), and the grain contained 35 to 38% moisture at that time.

Moisture content of the grain was higher than that of the cob in corn harvested soon after silking by Shaw and Thom (12). This relationship changed as the average moisture level reached 65 to 75% and cob moisture then exceeded grain moisture. The maximum difference in moisture per cent between the grain and cob averaged 23 to 24%, and occurred at approximately 35% moisture in the grain. Moisture per cent in both the cobs and husks of corn was observed by Aboul-Ela (1) to be higher than

kernel moisture, when kernel moisture was between 50 and 65%. As plants approached maturity, moisture percentage then declined rapidly in the husks and slowly in the cobs in comparison with kernel moisture.

### EXPERIMENTAL PROCEDURE

Four varieties and two hybrids which differ considerably in maturity and in growth characteristics, particularly in compactness of the head, were studied. For brevity of presentation, the varieties and hybrids will be referred to collectively as entries. The variety Norghum is early maturing and has an open or loose head type. Likewise, the De-Kalb C44a hybrid has an open head, but the head is considerably larger and neither blooms nor matures as early as Norghum. Martin and Midland are compact-headed varieties of midseason maturity. The head of Martin has a central stem or rachis which tends to become dry and brittle as the plant approaches maturity, while the rachis of Midland remains green as the grain and other plant parts mature. The RS610 hybrid is a moderately compact-headed type of approximately the same maturity as Midland and Martin. Double Dwarf Yellow Milo has a very compact, club-like head which does not open appreciably as it approaches maturity. Representative heads of the six entries are shown in Figure 1.

Five plantings were made during the 1958 spring season at Ames, Iowa, using a randomized block design, with three replicates of the six entries planted at each date. Planting dates ranged from May 22 to June 24, with each plot consisting of six rows 25 feet in length. All plots were thinned to a stand of six to eight plants per foot in rows spaced 40 inches apart. Thus tillering was minimized and the main tillers used for plant part separations could be selected with a minimum of difficulty.

Date mid-bloom was used as an aid in selecting plots of all entries which would reach a grain moisture level of approximately 45 to 50% at the same time. To further classify plants of equal development a system similar to the one described by Jensen (6) for marking plants with colored spray paints was used. Exceptionally early or late blooming plants were marked on the peduncle with paint and not used in sampling the plots. One of the six rows in each plot was used for grain moisture determinations prior to the initial harvest. The remaining five rows were divided into two sections and one of the ten sub-plots used at random to obtain samples at each of the ten harvest dates.

The initial harvest was made when the grain moisture of all entries was between 47.5 and 52.5%. Harvesting was initiated on September 12 and continued at five-day intervals through ten harvest dates, concluding on October 28. Ten uniform, primary-stalk plants per replicate were selected from each entry at each harvest date. All harvests of Norghum were made from the third planting (June 10), while samples of other entries were taken from the first planting (May 22). One replicate at a time was harvested for all entries, and plant part separations were made in approximately one hour to minimize moisture loss prior to weighing. Each plant was separated into grain, pedicles, rachis, peduncle, leaves, and stalk. A representative separation of the grain, pedicles, rachis, and peduncle is shown in Figure 2.





Figure 1. Representative heads of four grain sorghum varieties and two hybrids evaluated for moisture loss from plant parts. Threshed heads on left, normal heads on right.

(1) Norghum	(2) DeKalb C44a	(3) Martin
(4) Midland	(5) R.S. 610	(6) Double Dwarf Milo

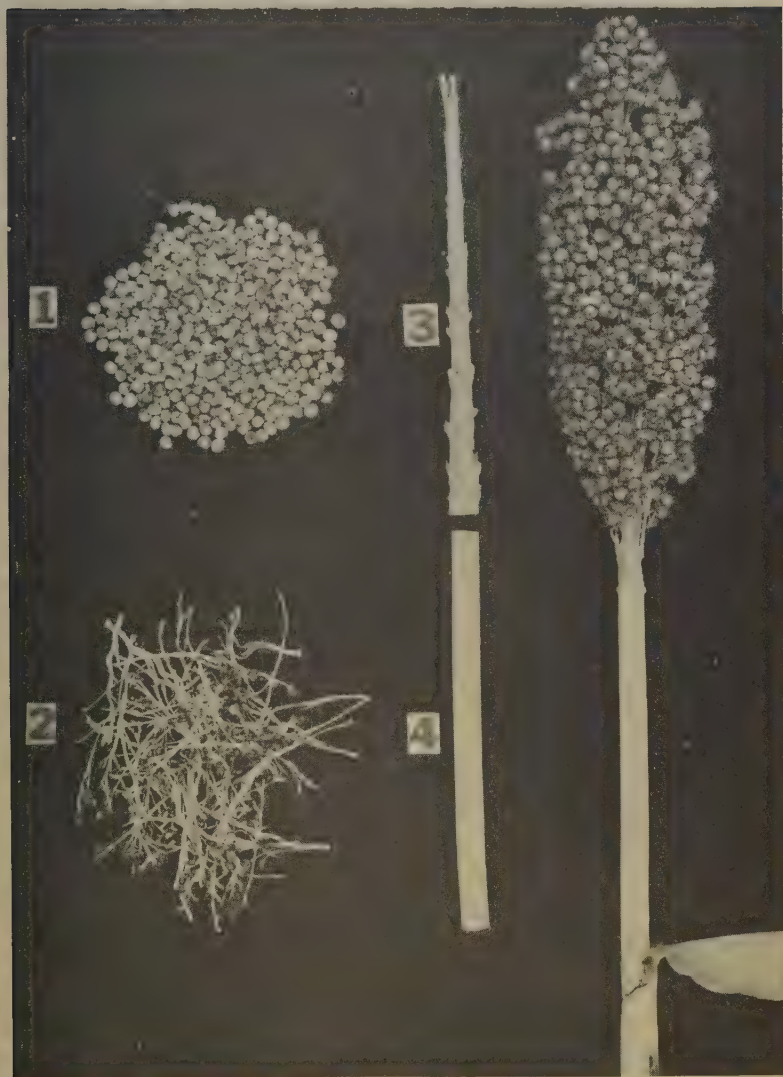


Figure 2. Plant part separation of the grain sorghum head.  
(1) Grain (2) Pedicels (3) Rachis (4) Peduncle

Heads were detached from the plant at the flag leaf and grain removed by placing the head in a Vogel head thresher operated at a slow cylinder speed. After separation of the grain the remaining outer glumes on a head were scraped from the pedicles. Pedicles were cut or pulled from the central rachis and the rachis cut at the base of the head to obtain the rachis and peduncle sections. Leaves were detached from the stalks at the leaf sheath attachment and stalks cut into lengths convenient for weighing and drying. The entire sample of each plant part was used in determining moisture percentages.

Samples of all plant parts were weighed to the nearest .5 gram, dried at 60°C for 96 hours, then moved to an electric drying oven at 100°C for 48 hours. This treatment was adequate to remove all moisture from the samples and moisture percentages at harvest were calculated from recorded moist and dry weights.

Duplicate 100-kernel samples from each plot for the first six harvests were weighed to determine the harvest moisture at which maximum dry weight of the grain was attained. All entries had reached 15 to 20% grain moisture at the sixth harvest date and kernel weights were not determined for later harvests. Kernel samples were weighed to the nearest one-hundredth gram and total weight of the 200 kernels used in analyses of the data.

Analyses of variance for all characters measured and regression analyses for part of the determinations were calculated using the methods outlined by Snedecor (13).

## RESULTS AND DISCUSSION

Climatic conditions during the 1958 season at Ames were favorable for good plant development and high grain yields for all full season crops. Moisture was adequate and temperatures relatively low as the sorghum plants developed toward bloom stage. Temperature and moisture conditions were exceptionally favorable for plant maturation and drying of the grain from the initial to the final harvest. Daily maximum temperatures for the September 12 to October 28 period averaged 10 to 15° above the comparable average temperatures recorded at Ames for the 25-year period 1931-1955, and total precipitation during the harvest period was only 1.57 inches. Of this total, 1.11 inches occurred two days after the initial harvest and only a trace to approximately .1 inch fell at any one time during the remainder of the harvest period.

The first frost of the fall season at Ames occurred on October 1 when a temperature of 29°F was recorded. A large majority of the leaves on all plantings were killed at this time, but stalks remained green and succulent. Subsequent frosts occurred on October 11 and 28 when temperatures of 29 and 30°F, respectively, were recorded. These frosts resulted in additional killing of leaves, but stalks still remained green and succulent. The October 1 frost occurred one day prior to the fifth harvest, and a marked increase in rate of moisture loss was observed after the fourth harvest for all plant parts except the grain and stalks. Thus for the pedicles, rachis, peduncles, and leaves the rates of moisture loss followed quite different patterns before and after the first frost.

The pattern for moisture loss from each plant part generally was similar for all varieties and hybrids. Moisture percentages for the various plant parts at successive harvests are shown in Figure 3 for the variety Norghum, and generally are representative of the trends observed for other entries. The most pronounced deviation from the patterns displayed for Norghum was observed among the grain moisture values obtained for Double Dwarf Milo after the third harvest. A comparison of mean squares for Dwarf Milo versus the other entries, through an F test, showed that this variety differed significantly from the other entries in rate of moisture loss from the grain, rachis and peduncles. Lack of conformity of the grain moisture determinations for Dwarf Milo as shown in Figure 4 probably can be attributed to extensive corn borer damage which occurred in all plots of this variety. Infestation by the European corn borer, *Pyrausta nubilalis* (Hbn.) was observed in nearly every plant of the Dwarf Milo plots, while practically none occurred in plots of the other entries. Borer damage was from second brood infestation and was expressed almost entirely as peduncle cavities in the area of the flag leaf attachment. It seems likely that this type of damage could materially affect translocation of water and nutrients to plant parts above this area.

Moisture content for both the stalks and peduncles of Norghum, shown in Figure 3, averaged approximately 80% at the initial harvest. These plant parts retained essentially the same moisture content through the first five harvests, or lost moisture at a slow rate. Moisture loss from the stalks continued at a slow rate throughout the entire sampling period, and all entries were within 3 to 6% of the initial stalk moisture at the last harvest. Loss of moisture from the peduncles of Norghum, and other entries, was greatly accelerated following the October 1 frost.

Leaf and rachis moisture content ranged from 72 to 77% for different entries at the initial harvest. Moisture was lost from both the leaves and rachis at a moderate rate prior to the frost, and again a marked increase in water loss was observed after the freeze. This was particularly true for the leaves, where the moisture loss was strikingly large and measurable soon after the freeze. Per cent of moisture in the leaves of Norghum was reduced approximately one-half between harvest dates four and five, with similar though somewhat less pronounced losses observed for other entries.

The pedicles and grain of Norghum approached 50% moisture at the first harvest, with moisture content ranging from 47 to 55% among all entries when sampling was initiated. Moisture content of the pedicles generally was 2 to 3% higher at the first harvest than was grain moisture. The pattern apparent for leaves, peduncles and rachis, wherein moisture loss was much greater following the frost, again was observed for the pedicles. Grain moisture losses were relatively rapid and uniform for most entries through the first seven harvest periods, both before and after the October 1 frost.

Sorghum grain may normally be expected to attain a minimum moisture content of about 12 to 13% on mature plants in this area, when favorable drying conditions occur. Thus the rate of moisture loss in the grain for all varieties and hybrids will of necessity slow down as this moisture content is approached. In this study all entries were closely approaching the 12 to 13% grain moisture level at the seventh harvest, and statements



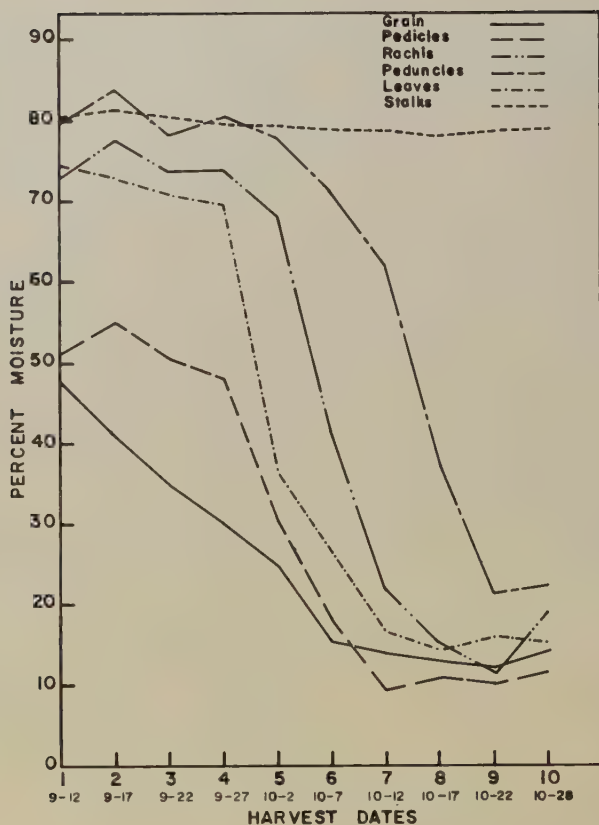


Figure 3. Percent of moisture at successive harvest dates for six plant parts of Norghum.

relative to grain moisture will be confined primarily to the first seven periods. For the other plant parts an equilibrium or leveling off point was not as definitely established, and data from all ten harvests was used in the interpretations for these characters.

Some fluctuations in the uniform downward trend in grain moisture percent for the six entries at successive harvest dates can be seen in Figure 4. However, the pattern was essentially linear through the first seven harvests for all entries, except the Double Dwarf Milo. The slight increase in grain moisture content observed at the tenth harvest may have resulted from a light rain the evening preceding this harvest and a white frost on the morning samples were taken.

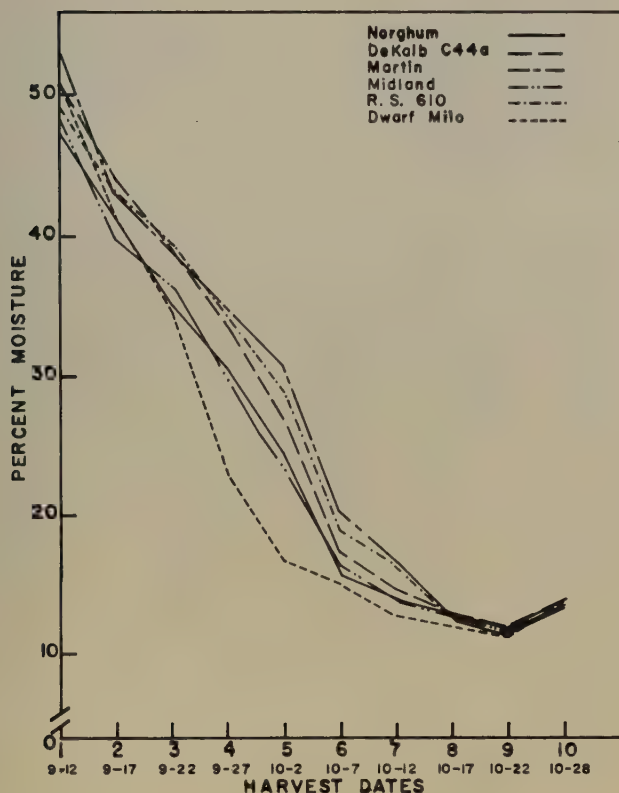


Figure 4. Grain moisture percent at successive harvest periods for six grain sorghum varieties and hybrids.

Regression analyses of grain moisture percentage on harvest date are presented in Figure 5. Data from the Dwarf Milo samples were excluded and only the first seven harvests were used for these calculations. Regression coefficients for all entries were grouped closely with a range from -5.62 to -6.54 obtained. An F test for significance of the differences among entry regression did not exceed the 5% level of probability, indicating no real differences were obtained in rate of moisture loss from grain of the five entries. The mean square associated with deviations from individual entry regressions accounted for a large portion of the total variation and it was not possible to establish significant differences among entry regressions.

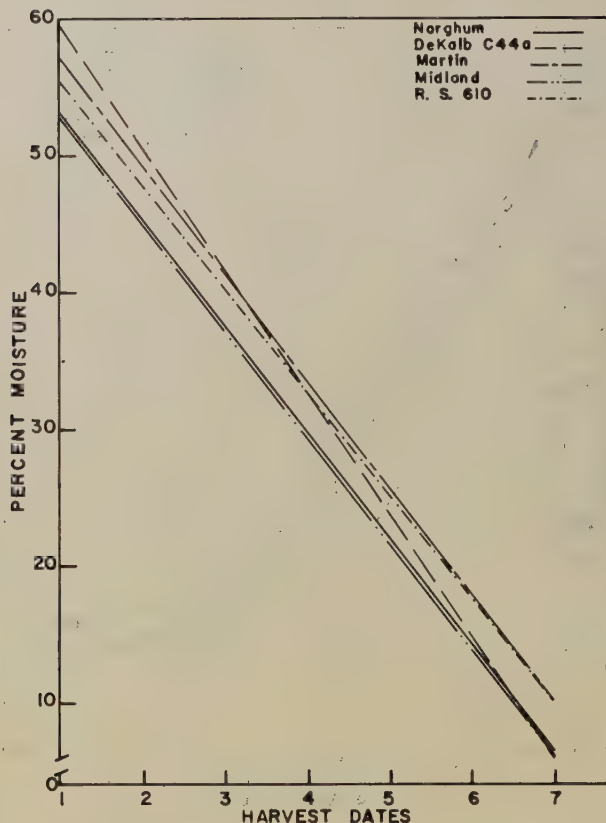


Figure 5. Regression of grain moisture percentage on harvest date for five grain sorghum varieties and hybrids.

A pronounced difference in rate of moisture loss from the rachis prior to and after the October 1 frost is shown in Figure 6. Moisture content decreased at a slow rate prior to the frost and then dropped rapidly. A similar pattern for moisture loss was observed for the pedicle, peduncle, and leaf samples. An increase in moisture content generally was observed for all plant parts except the grain, between the first and second harvests. Precipitation of 1.11 inches three days prior to the second harvest may have been a factor in effecting this increase. Light rain and frost prior to the final harvest also was a likely cause for the gain in moisture generally observed between harvests nine and ten.

Analyses based on linear or curvilinear regression methods did not seem appropriate for evaluating differences in moisture loss for data

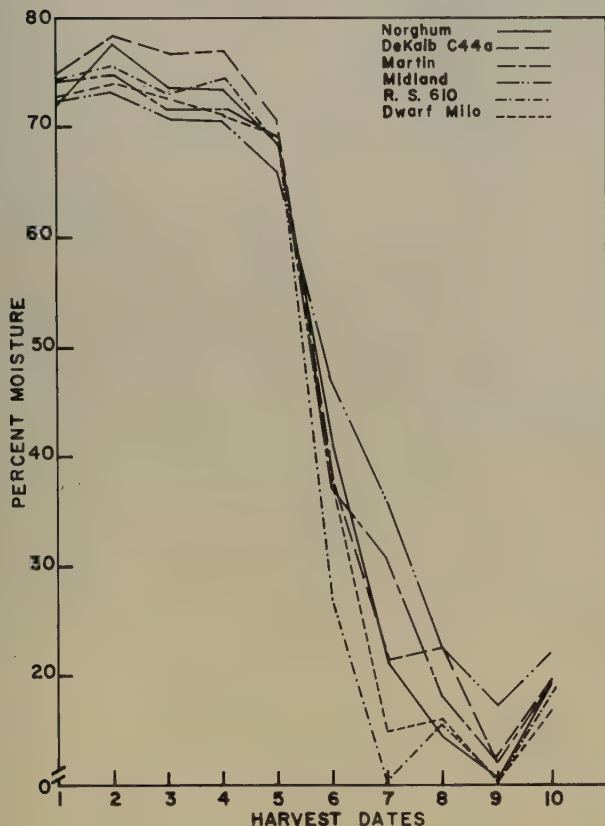


Figure 6. Rachis moisture percent at successive harvest periods for six grain sorghum varieties and hybrids.

obtained from the rachis, pedicle, peduncle, and leaf samples. An analysis of the differences in percent moisture between adjacent harvest dates seemed most appropriate for evaluating the relative rate of moisture loss among varieties and hybrids, and nine separate analyses for contiguous harvest dates were calculated for each character. In several instances significant differences obtained for the gain or loss in moisture between contiguous harvests could be explained either by rains which fell prior to harvest, or by deviation of the corn borer infested Dwarf Milo plots from the pattern observed for other entries. When these comparisons are excluded from consideration, the differences among entries for

rate of moisture loss exceeded the 5% level of probability in only seven of fifty-four comparisons between contiguous harvests. Thus for the pedicle, rachis, peduncles, and leaves all entries generally followed similar patterns of moisture loss.

The only appreciable difference in rate of moisture loss among entries was observed with the peduncle moisture determinations. After the frost Midland lost moisture at a considerably slower rate than other hybrids or varieties. At the final harvest it was approximately 16% higher in moisture than Norghum, more than twice as high as all other entries, and nearly three times greater in peduncle moisture than Dwarf Milo and RS610. Norghum likewise retained a relatively high peduncle moisture throughout the sampling period, though it was one of the lowest in grain moisture content. Peduncles of Midland and Norghum also retained a markedly greener color than other entries. However, the apparent greenness and high moisture content observed for the peduncle of these entries was not reflected in the grain moisture determinations. A further indication that peduncle moisture may have little association with grain moisture content was observed with the RS610 hybrid. This entry was among the lowest for peduncle moisture throughout the sampling period, but was one of the highest in grain moisture content at most harvest dates.

None of the freezing temperatures which occurred during the harvest period was severe enough to kill the stalks and apparently did not affect water movement in this segment of the plant. Average stalk moisture content for all entries, shown in Figure 7, decreased only 5.5% during the entire sampling period. Regression analyses for the stalk moisture percentages are presented in Figure 8. Regression coefficients ranged from  $-0.769$  for Dwarf Milo to  $-0.293$  for Norghum. Differences among entry regressions, as determined by an F test, exceeded the 1% level of probability. However, significance of the differences in moisture loss among entries likely is of little consequence in view of the low total moisture loss through the entire sampling period.

Grain moisture content of the various entries when maximum dry weight was attained fell within a range of 30 to 39%. Most entries decreased somewhat in 200-kernel weight as subsequent harvests were made at lower grain moisture levels, but the decreases generally were small and some deviations from this trend occurred. The grain moisture content of Norghum and Midland when maximum dry weight was attained, given in Table 1, generally was somewhat lower than that for other entries. Maximum dry weight for the other entries was reached at approximately equal moisture levels, though the DeKalb C44a hybrid generally contained slightly higher grain moisture than the remaining entries when maximum dry weight was obtained.

Ranges obtained for per cent moisture at the time maximum dry weight of the grain was attained compared favorably with the 35 to 38% moisture content reported by Clegg *et al.* (3), though the total range including all entries was somewhat greater. However, the period between mid-bloom and attainment of maximum dry weight of the grain varied from 40 to 46 days in this study, as compared with the 30-day period observed for the determination made in Nebraska.



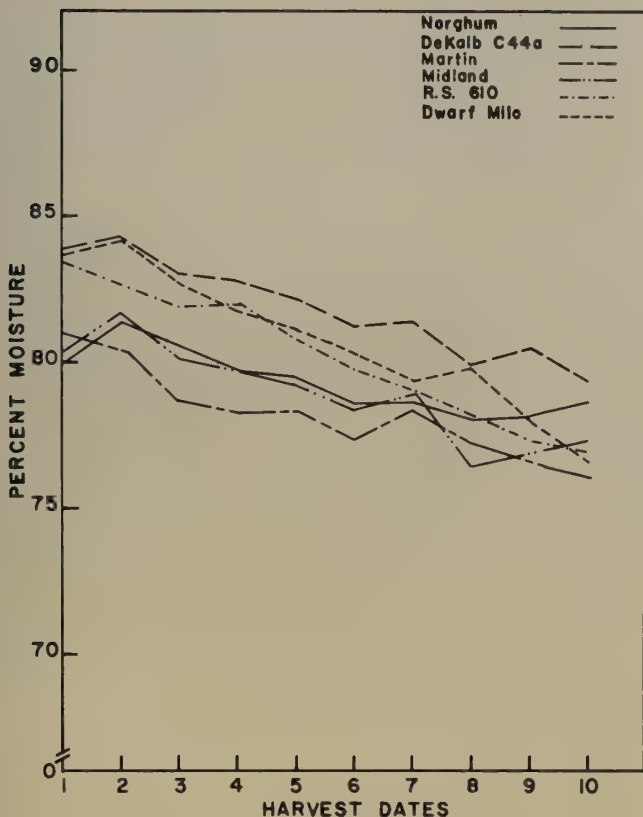


Figure 7. Stalk moisture percent at successive harvest periods for six grain sorghum varieties and hybrids.

#### SUMMARY AND CONCLUSIONS

Loss of moisture from the grain, pedicles, rachis, peduncle, leaves, and stalk of four grain sorghum varieties and two hybrids was studied through a period of ten successive harvests at five-day intervals. The initial harvest was made when grain of all entries was within a range of 47.5 to 52.5% moisture. Plant part separations were made for the varieties Norghum, Midland, Martin, and Double Dwarf Yellow Milo, and for the hybrids RS610 and DeKalb C44a. These entries differed considerably in relative maturity and growth characteristics, and particularly in compactness of the head.

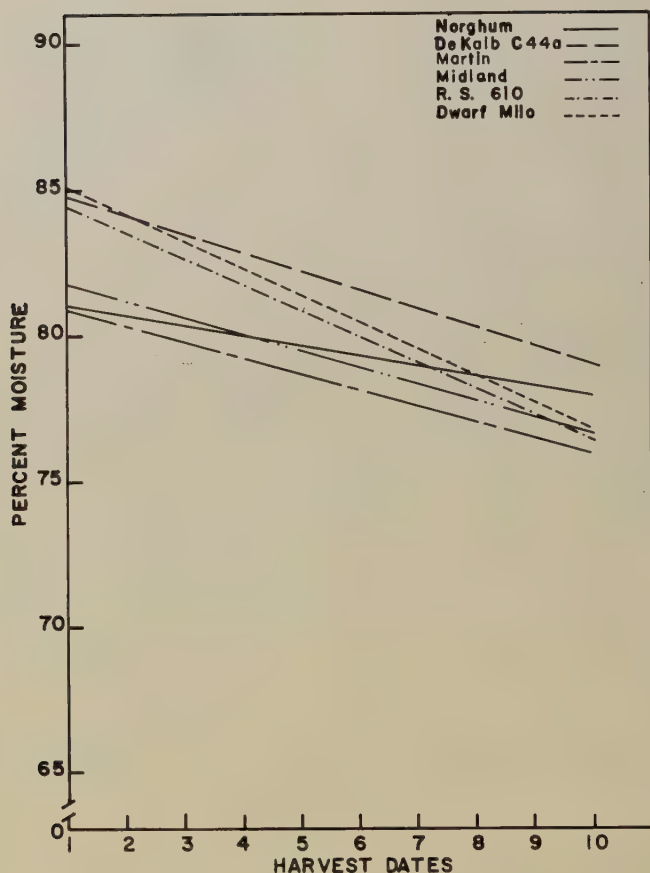


Figure 8. Regression of stalk moisture percentage on harvest date for six grain sorghum varieties and hybrids.

Rate of water loss from the pedicles, rachis, peduncle, and leaves was affected materially by the occurrence of frost and a 29°F low temperature two days prior to the fifth harvest. Moisture determinations for the grain and stalks were not influenced appreciably by the frost. Nearly all plants of Dwarf Milo were severely infested by the European corn borer at the base of the peduncle and results obtained for plant parts above the area of infestation may not have been typical of the normal performance for this variety.

Table 1. Range in moisture per cent at maximum dry weight of the grain for six grain sorghum hybrids and varieties.

Variety or Hybrid	Per cent moisture at maximum dry weight
Norghum	31-35
DeKalb C44a	34-39
Martin	35-38
Midland	31-35
RS610	34-39
Dwarf Milo	34-39

Loss of moisture from the grain of all varieties and hybrids was comparatively uniform and followed a linear pattern through the first seven harvests. Data obtained for Dwarf Milo deviated from the pattern observed for other entries, and it appears likely that the corn borer damage observed in this variety may have limited translocation of water and nutrients to the head and resulted in premature drying of the grain. A regression analysis of moisture percentages at the first seven harvest dates for the other entries indicated that rate of water loss among these entries did not differ significantly. Grain moisture content of all entries tended to level off at 12 to 13% after the seventh harvest and data from the last three harvests were excluded from the regression analyses.

Moisture loss from the stalks was slow and the average moisture content for all entries decreased only 5.5% through the entire harvest period. A regression analysis indicated that varieties and hybrids differed significantly in rate of moisture loss, but the importance of these differences likely is not great in view of the low moisture loss for the entire harvest period.

The pattern of moisture loss from the pedicle, rachis, peduncle, and leaves of all entries was similar, and was divided into three pronounced segments. Rate of water loss prior to the frost generally was slow and similar for all entries. After the frost, moisture was lost at a rapid rate until the seventh or eighth harvest, and a slower rate of loss then was observed through the final harvests. Analysis of the differences between moisture percentages at adjacent harvests gave a low proportion of differences which exceeded the 5% level of probability, and it was concluded that the hybrids and varieties evaluated did not differ appreciably in rate of water loss from the pedicles, rachis or leaves. Midland and Norghum lost moisture from the peduncle at a considerably slower rate than did the other entries.

A consistent association of rate of moisture loss from the grain with that from other plant parts was not established for any of the varieties or hybrids. Similarly, a consistent relationship between compactness of the head and rate of moisture loss from the grain was not observed. Entries which differed in degree of head compaction also were of different genetic constitution, thus a critical evaluation of rate of water loss in open versus compact headed types was not possible.

Maximum dry weight of the grain was attained when grain was harvested at between 31 and 39% moisture. Norghum and Midland attained maximum dry weight of the grain when grain moisture content at harvest was between 31 and 35%. A range of 34 to 39% grain moisture at harvest produced maximum dry weight of the grain for the other entries.

The results and conclusions presented were obtained in a season which was very favorable for growth and maturation of the sorghum plant. They may or may not be indicative of the general pattern of moisture loss from sorghum plant parts. Rates of moisture loss from the various plant parts, and interrelationships among them might differ considerably in a less favorable growing season or when harvests were made during a cool, humid fall season.

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# NUCLEOTIDE-DEPENDENT CARBON DIOXIDE FIXATION IN RHODOSPIRILLUM RUBRUM<sup>1</sup>

D.S. Bates and C.H. Werkman

Heterotrophic carbon dioxide fixation, first reported by Wood and Werkman (1), has been explained as a carboxylation of pyruvate resulting in the formation of oxalacetate. Subsequent investigations led to the suggestion that a phosphate ester might be involved in this carbon dioxide fixation (2). This view was emphasized when attempts to carboxylate pyruvate directly failed, whereas exchange of  $C^{13}O_2$  for  $C^{12}O_2$  occurred in the carboxyl adjacent to the methylene group of oxalacetate (3, 4). When the energy requirements of the reaction were evaluated, it became more probable that a phosphate ester was involved (5).

That such a phosphate ester is involved in carbon dioxide fixation was established by the discovery of two enzymes which catalyze the fixation of carbon dioxide into phosphoenolpyruvate (PEP\*) to form oxalacetate (OAA). One is phosphoenolpyruvic carboxylase (6), which catalyzes the irreversible reaction 1; the other is the nucleotide-requiring oxalacetic carboxylase (7), which catalyzes the reversible reaction 2.



Enzyme preparations catalyzing reaction 2 have been obtained from chicken liver (7), lamb liver (11), wheat germ (8), Thiobacillus thiooxidans (9), Nocardia corallina, Mycobacterium phlei and Rhodospirillum rubrum (10).

This report describes properties of the nonphotosynthetic, nucleotide-independent carbon dioxide fixation catalyzed by extracts of the photosynthetic autotroph, R. rubrum.

## MATERIALS AND METHODS

All chemical compounds were obtained from commercial sources.

Crude cell extracts of Rhodospirillum rubrum ATCC 11170 were prepared by sonic disruption as previously described (10), except that

<sup>1</sup> This work was supported in part by the U.S. Public Health Service.

\*Abbreviations: PEP, phosphoenolpyruvate; IDP, inosine diphosphate; GDP, guanosine diphosphate; UDP, uridine diphosphate; ADP, adenosine diphosphate; CDP, cytidine diphosphate; ITP, inosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate; ATP, adenosine triphosphate; OAA, oxalacetate; Tris, tris (hydroxymethyl)aminomethane; GSH, reduced glutathione; GSSG, oxidized glutathione; PCMB, p-chloromercuribenzoate;  $P_i$ , inorganic phosphate.



the washed cells were suspended in 5 volumes of 0.1 M Tris, pH 7.4, and no cysteine was added.

The crude extracts were partially purified by two methods. In the first method, the crude extract was stored about two months at  $-20^{\circ}\text{C}$  until used, made 10% by volume with saturated  $(\text{NH}_4)_2\text{SO}_4$ , and centrifuged 10 min at  $24,500 \times g$  at  $4^{\circ}\text{C}$ . The supernatant solution was dialyzed one hour against distilled water, then against 0.005M phosphate buffer, pH 5.9, one hour in an EC electroconvection apparatus at 40 volts. In the second method, the fresh extract (not stored) was treated as described above, then further fractionated with saturated ammonium sulfate. The 40-70% fraction was collected by centrifugation as described above, dissolved in the same volume of 0.9% KCl as the original volume of crude extract, and treated in the EC electroconvection apparatus. All operations were performed at  $4^{\circ}\text{C}$ .

The reactions were conducted and the reaction mixtures analyzed as previously described (10). The experimental error was about 5%.

Radioactive OAA was recovered as the 2,4-dinitrophenylhydrazone which was identified by paper chromatography and radioautography. The location of the radioactive carbon was determined by the method of Bandurski and Greiner (6).

Protein was determined by the biuret method (12) following the removal of cell pigments (13).

## RESULTS AND DISCUSSION

The results herein presented were obtained with extracts prepared by the first method except where indicated. Similar results were obtained with both extracts in a given experiment. OAA was the only compound which was significantly radioactive and was identified from reaction mixtures originally containing either PEP and IDP (fixation reaction) or OAA and ITP (exchange reaction).

That the reaction depended upon either PEP and IDP or OAA and ITP is indicated in Table 1.

Concentration effects of the various components are shown in Table 2. The activities either remained constant or decreased after the maxima were reached. Relatively small amounts sufficed for the attainment of the nucleotide maxima, whereas large bicarbonate concentrations were required.

Table 3 shows the effects of pH, time, and volume of extract. The pH optima were quite broad in both cases and the optimum in the fixation reaction appeared to be slightly more acidic. A linear relationship existed between the activity and reaction time as well as between activity and volume of extract.

The results of various nucleotide substitutions are shown in Table 4. Inosine and guanosine nucleotides appeared to be interchangeable. Apparently this system differs from that in *Saccharomyces cerevisiae* which requires ATP (14). It is not known whether both inosine and guanosine nucleotides satisfied the nucleotide requirement or one was converted to the other by some other system in the extract. Although not conclusive, experiments indicated little or no nucleoside diphosphokinase activity in these extracts.

The divalent metal ion requirement was satisfied by  $Mn^{++}$  to a much greater extent than by  $Mg^{++}$  or  $Co^{++}$  as indicated in Table 5.

The stimulation of the reaction by sulfhydryl compounds depended on the age of the extract. The reaction was stimulated somewhat by cysteine when freshly prepared extracts were employed, but the enzyme was active in the absence of added cysteine. However, old extracts required a sulfhydryl compound. Table 6 gives results of sulfhydryl compounds and also the inhibitory effect of a sulfhydryl inhibitor. One-tenth  $\mu$ mole p-chloromercuribenzoate (PCMB) in contact with the extract for 15 min completely inhibited the reaction, whereas the addition of 10  $\mu$ moles of cysteine restored some of the activity.

In preparing the extracts, fractionation or storage was necessary since the endogenous fixation in the absence of added nucleotide occurred in both the fixation and exchange reactions when crude extracts were employed. This fixation was not removed by various methods of dialysis, charcoal treatment, or ion exchange resins. It is not known whether this endogenous fixation was due to the irreversible phosphoenolpyruvic carboxylase (6); some unknown factor, enzymatic or nonenzymatic; or simply the failure to remove the nucleotides.

Preliminary experiments indicate that reaction 2 is also catalyzed by extracts of R. rubrum grown aerobically in the dark.

Table 1. Dependence of  $CO_2$  fixation on OAA and PEP.

Reaction mixtures contained, in micromoles: phosphate, pH 6.5, 50;  $MnCl_2$ , 5;  $NaHC^{14}O_3$ ,  $2.5(1 \times 10^7 \text{ cpm})$ ; cysteine, 10; substrate and nucleotide additions, 3, where indicated. Extract, 0.1 ml (125  $\mu$ g protein). Total volume 2.0 ml. Reaction time, 45 min. Gas phase  $N_2$ .

Additions	cpm/50 $\mu$ l*
OAA	7
ITP	4
OAA, ITP	632
PEP	5
IDP	0
PEP, IDP	219
Pyruvate, ITP	0
Pyruvate, IDP	0
Pyruvate, ATP, IDP	2
Malate, ITP	2

\* Counts per minute for 50  $\mu$ l of reaction mixture.

Table 2. Concentration effects.

Reaction conditions were the same as Table 1 except where varied as indicated.

Fixation reaction		Exchange reaction	
$\mu\text{moles}$	cpm/100 $\mu\text{l}$	$\mu\text{moles}$	cpm/100 $\mu\text{l}$
PEP		OAA*	
0.0	0	0.0	6
0.3	188	0.3	362
1.5	231	3.0	636
3.0	301	6.0	622
6.0	292	9.0	714
12.0	250		
IDP*		ITP*	
0.0	9	0.0	2
0.3	522	0.15	564
0.75	598	1.5	1148
1.5	596	3.0	670
3.0	556	4.5	398
4.5	326		
MnCl <sub>2</sub>		MnCl <sub>2</sub> **	
0.0	2	0.0	4
0.5	77	0.5	42
1.25	128	2.5	544
2.5	234	5.0	524
5.0	257	10.0	458
10.0	221		
Cysteine		Cysteine	
0.0	19	0.0	288
2.5	161	2.5	822
5.0	190	5.0	1002
10.0	275	10.0	1122
15.0	246	20.0	1032
20.0	276	25.0	982
NaHC <sup>14</sup> O <sub>3</sub> ***		NaHC <sup>14</sup> O <sub>3</sub> ***	
20	118	10	422
50	136	30	842
70	170	50	954
		60	1082
		70	1054

\*MnCl<sub>2</sub>, 2.5  $\mu\text{moles}$ ; NaHC<sup>14</sup>O<sub>3</sub>, 1.25  $\mu\text{moles}$ ; extract No. 2, 0.1 ml (66  $\mu\text{g}$  protein).

\*\*Cysteine, 5  $\mu\text{moles}$ .

\*\*\*Nucleotides, 1.5  $\mu\text{moles}$ ; MnCl<sub>2</sub>, 2.5; extract, 0.2 ml; reaction time, 60 min; NaHC<sup>14</sup>O<sub>3</sub> ( $4 \times 10^4$  cpm/ $\mu\text{mole}$ ).

Table 3. Effect of pH, reaction time, and extract volume.

Reaction conditions were the same as Table 1 except where varied as indicated.

Fixation Reaction		Exchange Reaction	
Reaction time		Reaction time	
min.	cpm/100 $\mu$ l	min.	cpm/100 $\mu$ l
15	88	15	574
30	142	30	882
45	151	45	1324
60	272	60	1574
Volume of Extract		Volume of Extract	
ml	cpm/100 $\mu$ l	ml	cpm/100 $\mu$ l
0.05	86	0.05	438
0.1	151	0.1	874
0.2	339	0.2	1634
pH*	cpm/100 $\mu$ l	pH**	cpm/100 $\mu$ l
6.0	186	5.7	920
6.2	250	6.0	1000
6.4	285	6.35	1036
6.6	281	6.6	1102
6.8	265	6.8	1160
7.0	241	7.0	1198
7.2	224	7.35	884
7.4	202	7.8	628
8.0	76	8.0	546

\*Phosphate, 60  $\mu$ moles

\*\*Phosphate, 100  $\mu$ moles

Table 4. Effect of various nucleotides.

Reaction conditions were the same as Table 1 except  $\text{MnCl}_2$ , 2.5  $\mu\text{moles}$  and nucleotide additions, 1  $\mu\text{mole}$ .

Exchange Reaction*		Fixation Reaction	
Additions	cpm/100 $\mu\text{l}$	Additions	cpm/100 $\mu\text{l}$
ITP	514	IDP	142
GTP	508	GDP	146
UTP	102	UDP	33
ATP	66	ADP	17
IDP	32	ITP	12
CTP	22	CDP	4
None	14	None	4

\*Reaction time, 60 min.

Table 5. Effect of various divalent metal ions.

Reaction conditions were the same as Table 1 except  $\text{NaHC}^{14}\text{O}_3$ , 1.25  $\mu\text{moles}$  ( $5 \times 10^6$  cpm) and ion additions, 5  $\mu\text{moles}$ .

Additions	Fixation Reaction* cpm/100 $\mu\text{l}$	Exchange Reaction cpm/100 $\mu\text{l}$
$\text{MnCl}_2$	146	818
$\text{CoCl}_2$	21	134
$\text{MgCl}_2$	12	120
$\text{FeCl}_2$	8	50
$\text{NiCl}_2$	2	8
None	2	8

\*Reaction time, 60 min.



Table 6. Effect of sulfhydryl compounds.

Reaction mixtures contained, in micromoles; phosphate, pH 6.5, 60;  $\text{MnCl}_2$ , 2.5;  $\text{NaH}^{14}\text{CO}_3$ ,  $1.25(5 \times 10^6 \text{ cpm})$ ; OAA (or PEP), 3; ATP (or IDP), 0.75; 0.2 ml extract; and additions as indicated in micromoles. Total volume, 2.0 ml. Gas phase,  $\text{N}_2$ . Reaction time, 60 min.

Additions	Exchange Reaction cpm/50 $\mu\text{l}$	Fixation Reaction cpm/50 $\mu\text{l}$
None	54	30
Cysteine (10)	1380	233
GSH(10)	1420	154
Cystine (6.5)	30	38
GSSG (10)	0	4
PCMB (0.1)	1	1
PCMB (0.1), Cysteine (10)	574	72

## SUMMARY

Evidence has been presented which indicates that cell-free extracts from light-grown cells of *Rhodospirillum rubrum* contain a reversible, nucleotide-requiring, carbon dioxide fixing system. Manganous ion appears to be the most efficient in satisfying the divalent ion requirement. Inosine and guanosine nucleotides are equally effective as cofactors. Sulfhydryl compounds produce a stimulatory effect. The optimum pH range appears to be from 6.4 to 7.0.

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## HERITABILITY PERCENTAGES AND DEGREES OF DOMINANCE FOR QUANTITATIVE CHARACTERS IN OATS<sup>1</sup>

K.R. Jones and K.J. Frey<sup>2</sup>

Progress in plant breeding depends upon obtaining genetic diversity and the effectiveness of selection. Several phenomena make the selection phase difficult, but chief among them are the confounding effects of variation caused by nonadditive gene action and environment. These sources of variation may and often do cause plants with mediocre genotypes to appear phenotypically superior or vice versa. Therefore, knowledge of the relative contributions of environmental and additive and non-additive genetic effects to variation among plants and their progenies would be useful in determining the proper selection procedure.

The investigations presented here gave estimates of the relative magnitude of the environmental and additive and dominance components of variation for the  $F_2$  and  $F_3$  generations of 20 oat crosses, for heading date, plant height, grain yield, and weight per 100 seeds. From the variances, heritability percentages, degrees of dominance, and expected genetic advances from selection were determined.

### REVIEW OF LITERATURE

The value of statistics in estimating the factors responsible for biological variation was proposed by Fisher (6) in 1918. Subsequently a number of statistical models were proposed to determine the components of variance for segregating populations (5, 15, 17, 30). These made possible the analysis of phenotypic variation for use in estimating heritability percentages.

Lush (21) classified heritability percentages into two categories: (a) broad, or the ratio of genotypic to total variance, and (b) narrow, or the ratio of additive genetic to total variance. According to Johnson *et al.* (19) a heritability percentage indicates the expected effectiveness of selection in retaining phenotypic performance. Similarly, Grafius *et al.* (16) and Bartley and Weber (1) suggested that the magnitude of the heritability values indicated the characters for which selection would be successful and the generation when selection could be profitably practiced. Burton (2) suggested the use of both heritabilities and genetic coefficients

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by variation to show the progress expected from selection. By using heritability values to predict the expected gain from selection in barley, Frey and Horner (10) obtained a high positive correlation between expected and actual gains.

Lindstrom (20) and Charles and Smith (3) showed that dominance was responsible for considerable variation in quantitative characters in tomatoes. Using a formula developed by Comstock and Robinson (5), Gardner *et al.* (14) found the degree of dominance for nine quantitative characters in corn to vary from partial to overdominance. Mather (22) utilizing data from Quisenberry (24) estimated dominance at 0.9 for grain length. Noll (23), Garber and Quisenberry (13), and Sappenfield (26) found earliness of heading date in oats to be dominant.

Wallace *et al.* (27), using data from the  $F_3$  and  $F_4$  generations of the oat cross Letoria x Fulwin, found genetic coefficients of variation of 13, 5, and 7% for grain yield, seed weight, and height, respectively. From parental and  $F_2$  variances of the same cross Wallace *et al.* (28) estimated the genotypic variance of the parents as 29% for yield and 4% for plant height. Frey and Horner (11), using the standard unit method, found the heritability for heading dates in 22 oat crosses to be 62, 63, and 68% for the  $F_2$ - $F_3$ ,  $F_3$ - $F_4$ ,  $F_4$ - $F_5$  comparisons, respectively.

Weibel (29), working with wheat, found heritability percentages of 50, 8, and 45 for plant height, yield, and kernel weight, respectively. In barley, heritabilities of 92% for heading date, 75% for plant height, 50% for yield, and 38% for kernel weight were obtained by Fiuzat and Atkins (7), and Frey (8) found a mean heritability of 76% for heading date. Grafius *et al.* (16) reported heritability percentages for yield of 9 and 35 for the  $F_2$  and  $F_3$  generations, respectively. Heritabilities of 39% for yield, 42% for plant height, and 93% for heading date in barley were reported by Frey and Horner (10), whereas Jogi (18) found heritability values of 62, 67, and 93% for these characters, respectively.

## MATERIALS AND METHODS

The materials for this study were 20 oat crosses involving 22 parental sources of germ plasm from Avena sativa and A. byzantina. In 1953, 150  $F_2$  seeds (from one  $F_1$  plant) from each cross were space-planted in the field at Ames, Iowa, and the remnant of the second-generation seeds was placed in cold storage. From each cross 75 randomly selected  $F_2$  plants were harvested and threshed separately. Each line arising from an  $F_2$  plant was carried in bulk for 2 subsequent generations: the  $F_3$  and  $F_4$  generations were grown one line per 2-foot row at Ames in 1954 and 1955, respectively.

For this study, conducted in 1956, each cross was represented by a random sample of 75 plants from the  $F_2$  remnant, and one plant from each of the 75  $F_2$  derived lines, then in the  $F_5$  generation. To provide an estimate of environmental variance, 45 and 30 seeds of Clintland and Mo. 0-205 varieties, respectively, were sown with each cross.

A plot was one row containing 15 single-plant hills from the same generation spaced 1 foot apart. The 15 plots per cross, 5 each from the  $F_2$ ,  $F_5$ , and pure-line varieties, were sown in a completely randomized

experiment measuring 15 x 15 feet. Border effects were eliminated by discarding the end plants of each plot and growing guard rows on both sides of an experiment area.

The data recorded for all 20 crosses were plant height in inches and yield of grain in grams per plant. In addition heading date (date when the first head on a plant was completely emerged) and kernel weight were determined for 10 crosses.

The mean variance of the varieties, used as an estimate of environmental variance, was subtracted from the phenotypic variance for each generation to determine the genotypic variance. This type of subdivision involved the following assumptions: (2) no epistasis, (b) no linkage, (c) normal diploid meiosis, (d) a gene frequency of 0.5 for segregating loci, (e) no multiple alleles, (f) no genotype x environment interactions, and (g) random selection. Assumptions c, d, e, and g were probably more completely fulfilled than were a, b, and f.

By using formulas derived by Grafius (15) and Horner et al. (17) for the genetic model entitled "additive model with dominance," it was possible to obtain coefficients of additive (D) and dominance (H) gene action. The  $F_2$  and  $F_5$  genotypic variances were equated to their respective D and H components as follows:

$$\text{I. } \sigma^2_{gF_2} = .5000 D + .2500 H$$

$$\text{II. } \sigma^2_{gF_5} = .9375 D + .0586 H$$

By solving the equations simultaneously estimates of D and H were obtained. The average degree of dominance was calculated as  $\left(\frac{H}{D}\right)^{\frac{1}{2}}$ .

Heritability percentages were calculated in the broad sense for the  $F_2$  and  $F_5$  generations and in the narrow sense for the  $F_2$ ,  $F_5$ , and infinite generations. The heritability percentages for the infinite generation were calculated from formula III.

$$\text{III. Heritability per cent} = \frac{D}{D + \text{error}} .$$

The denominator represented the theoretical phenotypic variance of the infinite generation assuming that (a) the environmental variance remained constant, (b) with continuous selfing the dominance variance disappeared, and (c) with each successive generation the additive genetic variance increased one-half of the increase in the previous generation. The broad heritabilities were obtained by dividing the total genotypic variance for a given generation by the total phenotypic variance. The narrow heritability percentages were determined by dividing the additive genetic by the phenotypic variance.

## EXPERIMENTAL RESULTS

### Dominance degree

The mean degree of dominance for grain yield (Table 1) indicated extreme overdominance. In 13 of the crosses the dominance degree was



Table 1. Average degree of dominance for 4 characters in oat crosses.

Character	Number of crosses	Average degree of dominance
Heading date	10	0.47
Plant height	20	0.91
Kernel weight	10	1.43
Grain yield	20	2.45

1.0 or greater. Studies by Gardner *et al.* (14) and Robinson *et al.* (25) on yield of corn also showed overdominance if repulsion linkages were absent. Overdominance was indicated for weight per 100 seeds, whereas the dominance degree for plant height and heading date were only partial.

Of speculative interest is the possible contribution of geometric epistasis (9) to the magnitude of degree of dominance. Heading date, which was a sum of 2 intervals, from planting until floral initiation and from floral initiation to heading date, showed incomplete dominance; plant height, which was a product of 2 measurements, the number of internodes and the mean length per internode, showed nearly complete dominance; kernel weight, which was a cubic function of width, length, and density, showed overdominance; and grain yield, which was a product of number of heads per plant, number of seeds per head, and kernel weight displayed extreme overdominance. Frey (9) pointed out the importance of geometric epistasis to the case of overdominance where the  $F_1$  hybrid yielded several hundred per cent more than either parent. Genotype x environment interaction can also be interpreted as overdominance. A genotype x environment interaction larger in the  $F_2$  than in the  $F_5$  could be responsible for indicating overdominance and negative additive variances, and conversely, a larger  $F_5$  interaction could cause negative dominance variances and underestimates of the degree of dominance.

Degree of dominance estimates do not relate the direction of dominance, i.e., whether negative or positive, but must be obtained by growing the parents with each cross. Other studies give some indication of the direction of dominance for the characters studied. Sappenfield (26) found earliness to be dominant, and height and yield of the  $F_1$ 's were greater than the midpoint between the parents in studies by Coffman and Stephens (4). Wallace *et al.* (27) found the  $F_2$  means for plant height, seed weight, and yield significantly greater than the midpoint of the parents.

#### Heritability

Frey *et al.* (12) explained that with successive generations of selfing narrow and broad heritability percentages should approach the same value. This conclusion can be substantiated in 2 ways: (a) relationship of

Table 2. Narrow and broad mean heritability percentages for the  $F_2$ ,  $F_5$ , and infinite generations of oat crosses.

Character	No. of crosses	$F_2$ generation		$F_5$ generation		Infinite generation
		Broad	Narrow	Broad	Narrow	
Heading date	10	60	56	70	69	71
Plant height	20	56	44	64	60	64
Kernel weight	10	52	38	55	49	52
Grain yield	20	41	11	30	22	25

mean heritability percentages, and (b) the correlations between narrow and broad heritability percentages in the  $F_2$  and  $F_5$ . In all cases, the mean broad and narrow heritability percentages were in closer agreement in the  $F_5$  than in the  $F_2$  generation (Table 2) as a result of the reduction in the contribution of nonadditive gene action inherent with selfing.

The correlations between the  $F_5$  broad and narrow heritability percentages were high for all 4 characters, whereas similar relationships in the  $F_2$  were lower and some were negative (Table 3). Both the mean heritability percentages and the correlations indicate that the narrow and broad heritability percentages approach the same value with successive generations of selfing. The broad heritability percentages for the  $F_2$  and  $F_5$  were in close agreement except for kernel weight.

The method used to estimate narrow heritability percentages required growing 2 segregating generations simultaneously, a procedure not practical in a breeding program. However, the data for calculating broad fertility percentages could be obtained easily from a practical breeding program and used to estimate the potential of a cross. In order to test the validity of broad heritability percentages they were correlated with the infinite generation heritabilities which should represent the maximum attainable values. The correlations between  $F_2$  broad and infinite-generation heritability percentages ranged from -0.07 to 0.64, whereas those between  $F_5$  broad and infinite-generation heritabilities were all +0.95 or higher, indicating that the  $F_5$  values would be nearly as reliable as the highest heritability percentages attainable.

#### Expected genetic advance

The magnitude of genetic advance from selection for a character in a cross is determined by two factors: (a) amount of genetic variability present, and (b) the heritability of the character. Even if the heritability was high, little progress could be made for selection unless considerable genetic variability was present. In other words the magnitude of genetic advance might be equal by selecting in a cross which exhibited low heritability and high genetic variability and in one with high heritability and low genetic variability. The expected genetic advances from selecting

Table 3. Correlations between the heritability percentages of the  $F_2$ ,  $F_5$ , and infinite generations of oat crosses.

Generation and method	Heading date	Plant height	Kernel weight	Grain yield
Broad $F_2$ and infinite	0.94**	0.64**	-0.07	0.45**
Broad $F_5$ and infinite	1.00**	0.96**	0.97**	0.95**
Broad $F_2$ and $F_5$	0.95**	0.80**	0.14	0.64**
Broad and narrow $F_2$	0.68**	-0.02	-0.69*	0.04
Broad and narrow $F_5$	0.99**	0.82**	0.90**	0.91**

the plants which exceeded the population mean by 2.06 times the standard deviation were calculated by using the following formula (19):

$$\text{Expected genetic advance} = 2.06 \left( \text{genetic coefficient of variability} \times \text{square root of heritability} \right).$$

The values obtained with the formula are expressed in percent of the population means and represent the genetic advance expected from one cycle of selection (Table 4). The expected gains for yield, the character with the lowest heritability, were 9.7 and 21.8%, in the  $F_2$  and  $F_5$ , respectively, while in contrast, the corresponding gains for heading date, a highly heritable character, were only 4.1 and 6.2%, respectively. The contrast between these two characters exemplifies the importance of both heritability percentage and genetic variability in the genetic advance attainable through selection. The use of heritability values alone to determine the generation in which to select for a character or to estimate the value of a cross would be questionable.

The expected genetic advance for yield ranged from 0 to 30% among crosses in the  $F_2$  and from 0 to 60% in the  $F_5$ . Values of 40 to 50% do not seem plausible in light of experience. Wallace *et al.* (27) predicted yield gains of 15% of the mean from selection of oat plants in the  $F_2$ .

For each character the mean expected gain was higher in the  $F_5$  than in the  $F_2$  for 2 reasons: (a) heritability percentages increased between the  $F_2$  and  $F_5$  (Table 3) and (b) segregation between the  $F_2$  and  $F_5$  generations resulted in greater genetic variability in the latter generation.

## DISCUSSION

Agronomic characters of economic importance are often subject to large "nonfixable" variations from environment and nonadditive gene action because they are complexly inherited. Permanent gain in a quantitative character from selection is inversely related to the extent of influence of nonfixable variation.

Table 4. Mean expected genetic advance (in percent of the character means) from selection for 4 characters in a number of oat crosses.

Character	Number of crosses	Expected genetic advance from selection in:	
		F <sub>2</sub>	F <sub>5</sub>
Heading date	10	4.1	6.2
Plant height	20	7.8	12.8
Kernel weight	10	9.7	15.0
Grain yield	20	9.7	21.8

Nonadditive gene action is due to dominance and epistasis, but the model used in this study disregarded the latter type. As suggested by Horner *et al.* (17) failure to take epistasis into consideration may cause an upward bias of the additive and dominance variance estimates. Another source of bias was the genotype x environment interactions. Wallace *et al.* (27) and Johnson *et al.* (19), respectively, found large genotype x location and genotype x year interactions for oats and soybeans.

For certain characters plants selected in spaced plantings would not produce the same expression that they would in competitive stands. Therefore, inferences on the basis of data collected from spaced plants would not necessarily be corroborated in subsequent progeny tests. For example, number of tillers per plant is highly correlated with yield in spaced plantings (7), but in a solid stand these two characters are virtually uncorrelated.<sup>3</sup> Consequently, a high tillering and thus high yielding spaced plant might produce a progeny of nominal yielding ability when grown in solid stand. Increased competition would influence heading date, plant height, and kernel weight less than it would yield; so data obtained from spaced plants for the first 3 characters might be applicable in solid-planted materials.

The dominance estimates obtained were similar to those reported by Gardner *et al.* (14) for corn, with the highest values being obtained for yield and its components. Since the frequency of the heterozygous phase of a locus in an oat population decreases by one-half with each successive generation due to selfing, any value from dominance is soon lost. Therefore, if a character exhibited considerable dominance variance, as was the case for yield, selection in the F<sub>2</sub> would not give as much permanent gain as expected, which fact suggested the desirability of postponing selection for yield until later generations. Such a procedure, however, would incur the carrying of large numbers of unprofitable progenies.

<sup>3</sup> Unpublished data from the Iowa Agricultural Experiment Station.

Deferring the selection until the  $F_5$  would increase the expected genetic gains by approximately 150, 150, 160, and 200% for heading date, height, kernel weight, and yield, respectively. These data support the use of the bulk breeding method, which permits carrying a large number of crosses into the later generations economically.

### SUMMARY

Data from plants of 2 oat varieties and the  $F_2$  and  $F_5$  generations of 20 oat crosses were used to calculate broad and narrow heritability percentages, the average degrees of dominance, and the expected genetic advances from selection for heading date, plant height, kernel weight, and yield.

The average degree of dominance for heading date was 0.47; for plant height, 0.91; for kernel weight, 1.43; and for yield, 2.45. The average heritability percentages were highest for heading date and plant height and lowest for yield. The correlations between  $F_5$  broad and infinite-generation heritability percentages ranged from 0.95 to 1.00 for the 4 characters.

Estimates of genetic advance from selection indicated that sizeable gains could be expected from selection in the  $F_2$  generation for any of the 4 characters. However, selection efficiency was increased considerably by delaying selection until the  $F_5$ .

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## THE EFFECT OF PLASTIC MULCH ON THE MICRO-CLIMATE AND PLANT DEVELOPMENT<sup>1</sup>

Leo J. Fritschen and R.H. Shaw<sup>2</sup>

Soil mulches of leaves, straw, and sawdust have long been used for weed control and moisture conservation on horticultural crops. Recently, increased yields and early maturity of various crops have been reported when plastic films were used as the mulch (2, 3, 5, 12). Very limited research has been conducted on the effect that these plastic films have on the micro-climate. Most of the micro-climatic research has been limited to soil temperature measurements (1, 8, 12). The soil temperature data which appear in the literature are for selected days and may not be representative of the complete growing season. These data usually were obtained from small plots without crops growing on them and may not be representative of conditions under actual crop growth.

The effect that plastic films have upon the energy budget has also received only limited attention. Energy measurements have been limited because of the cost of obtaining a continuous record and the lack of instruments available for measuring it. The recent development of small inexpensive net radiometers has made the study of energy relations on a field basis possible.

An experiment, which was primarily designed to measure the evapotranspiration components of corn on a field basis, was also designed to measure the effect of black plastic film upon the micro-climate and plant growth. Measurements of the micro-climate were limited to soil and air temperatures and net radiation.

### MATERIALS AND METHODS

The design of the experiment was a randomized complete block with two treatments and two replicates. The two treatments were: (1) corn growing under natural conditions; and (2) corn growing through a 6-mil plastic film (Vis-Queen) which covered the ground. These two treatments will be referred to as natural plots and plastic covered plots, respectively. Each of the plots was 40 feet square. The experimental area was planted to corn (Iowa Hybrid 4570) on May 15, 1959. The corn was planted in 40-inch hills at the rate of four plants per hill, or approximately 15,682 plants per acre.

A high soil moisture content was maintained on the two treatments by

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means of supplemental irrigation. The soil moisture content (4-foot profile) in the plastic covered plots was above 60% available 94% of the time, while the soil moisture content in the natural plots was above 60% available 97% of the time.

Soil and air temperatures and net radiation were measured in one plot of each treatment of the experiment. The measurements of soil and air temperatures and net radiation were replicated three times within these plots with the exception of the standard screen height and the crop surface height air temperatures which were not replicated because the instrument location was not considered as critical.

Soil and air temperatures were recorded hourly on a multipoint Brown strip chart potentiometer using copper constantan thermocouples from June 11 to September 9, 1959. Soil temperatures were measured at 6 mm and at 10 cm below the surface. Air temperature was measured at 5 cm, 30 cm, standard screen height, and crop surface height. Both soil and air temperature sensing elements were constructed similar to those described by Portman (10 and 11). The thermocouples located in the air temperature sensing elements were shielded from solar and terrestrial radiation.

Net radiation was measured 1 m above the crop surface and 6 inches above the soil or plastic surface within each treatment with 12 miniature net radiometers, Model II, which were constructed similar to Model I and Model III described by Fritschen and van Wijk (7) and Fritschen (6). The output from each of the 12 miniature net radiometers was recorded for a 2-minute period every 30 minutes on a single pen Brown strip chart potentiometer. These recordings were made continuously from June 11 to September 9, 1959.

## RESULTS AND DISCUSSION

### Temperatures

To illustrate the effect of the plastic film on the soil and air temperatures, the hourly temperatures were averaged for 5-day periods. The 5-day averages of hourly temperatures in the plastic plots were plotted for the period June 11 to September 9, 1959 and were analyzed by drawing isotherms. Thus the isotherms (Figs. 1-4) show diurnal and seasonal temperatures (soil and air) observed in the plastic covered plots. The shading in these figures, represents the temperature differences which occurred between the plastic covered and the natural plots. This temperature difference is positive when the temperature in the plastic covered plot is the greatest. The diurnal and seasonal temperatures (soil and air) for the natural plots can be estimated by subtracting the observed treatment difference from the isotherms presented. For example, the 10-cm soil temperature in the plastic covered plot was 95° at 1900\* for the period June 16-20 (Fig. 1). The shading indicated a 11-15° treatment difference. The soil temperature in the natural plots was between 80 and 84°; it actually was 84° at this time.

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\*The hours of the day are numbered consecutively from 0000 to 2300 with midnight represented by 0000. Thus 1900 is 7.00 p.m.

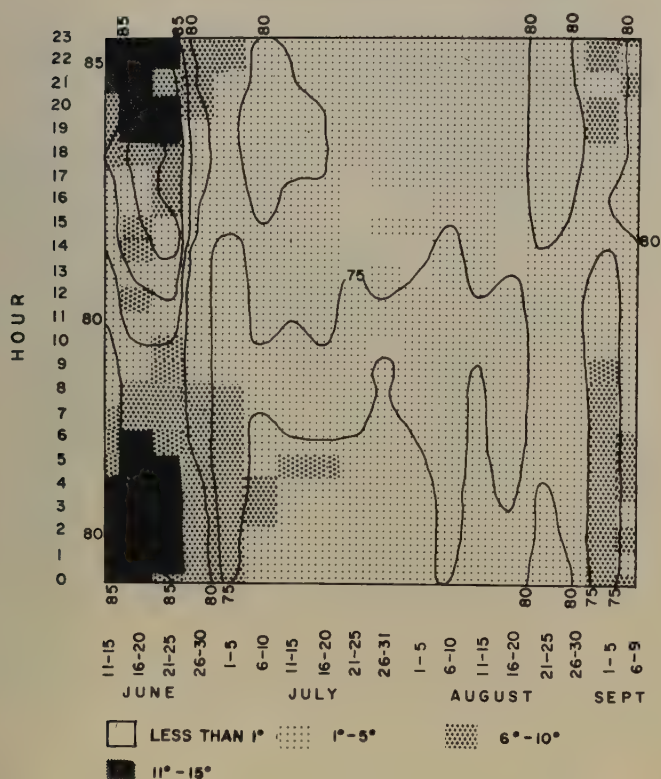


Figure 1. Diurnal and seasonal 10-cm soil temperatures for the plastic covered plots and the temperature difference between treatments.

### Soil temperatures

The soil temperatures observed at the 10-cm depth are shown in Fig. 1. The greatest range of diurnal 10-cm soil temperature occurred during June when the corn was small and did not greatly shade the ground. In general, the 5-day average diurnal temperature ranges were 1 to 6° greater in the natural plots than in the plastic covered plots.

The daily maximum temperatures, at the 10-cm depth, usually occurred at 1900 in the plastic covered plots and 1700 in the natural plots. The general time of occurrence of these maxima did not change during the period reported. The daily minimum temperatures, occurred at 0600 during June, at 0700 during July, at 0800 during August and September in the plastic covered plots. In the natural plots the daily minimum soil temperatures occurred at 0400 during June, at 0700 during July, 0800 during August and 0600 during September.



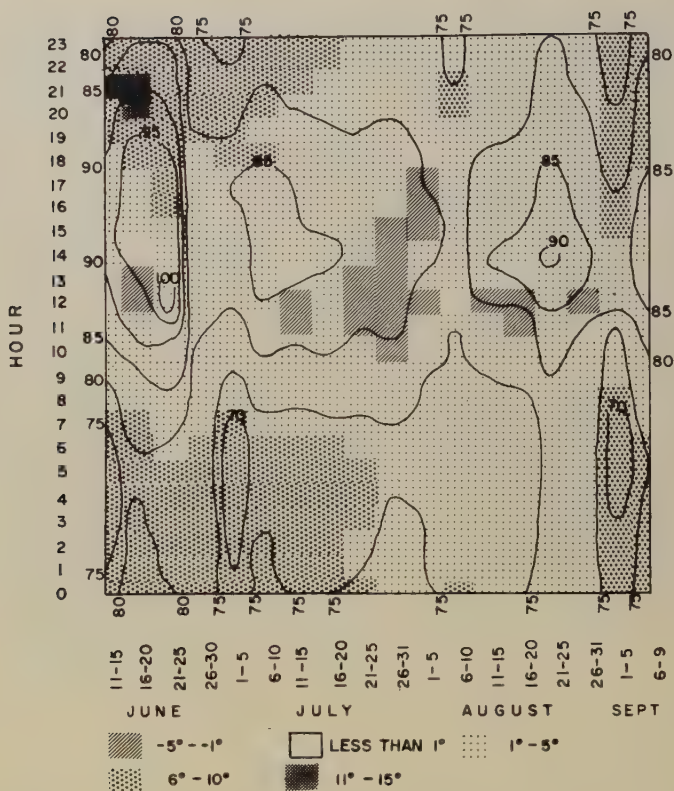


Figure 2. Diurnal and seasonal 6-mm soil temperatures for the plastic covered plots and the temperature difference between treatments.

The greatest temperature difference between the treatments occurred at night during early June (see shaded area, Fig. 1). In general, the difference in soil temperatures was less than  $5^{\circ}$  during July and August, and increased to less than  $10^{\circ}$  during September. The 10-cm soil temperatures were always greater in the plastic covered plots. The average seasonal temperature was  $78.2^{\circ}$  in the plastic covered plots and  $74.0^{\circ}$  in the natural plots.

The 6-mm soil temperatures are shown in Fig. 2. The diurnal temperature ranges, at the 6-mm depth, appeared to be correlated with the net radiation measured 6 inches above the surface. The following regression equations were computed for the natural and plastic covered plots, respectively:

$$\hat{Y} = 9.720 \ln X - 24.602, r = .90**,$$

$$\hat{Y} = 6.645 \ln X - 14.674, r = .81**,$$

where  $\hat{Y}$  is the estimated diurnal temperature range and  $X$  is the net radiation measured 6 inches above the surface. The correlation in the plastic covered plots was not as high as the natural plots because the plastic tends to dampen the diurnal range by causing a heat build-up under the plastic.

As one would expect, the daily maximum and minimum soil temperatures occurred earlier at the 6-mm depth than at the 10-cm depth in both treatments. The 6-mm maximum daily soil temperature generally occurred at 1600 to 1700 during June and at 1400 during the rest of the season in the plastic covered plots. In the natural plots, the daily maximum temperatures occurred at 1500 during June and at 1300 during the rest of the season. The 6-mm minimum daily soil temperatures occurred at 0500 hours, in both treatments, throughout the season.

The greatest treatment temperature differences were noted at night during the early and late portion of the growing season (see shaded area, Fig. 2). Here again, the plastic covered treatment had the highest soil temperatures. The smallest treatment temperature differences were observed during the midday hours. At this time, the soil temperature differences fluctuated around zero depending upon the synoptic situation. The treatment temperature differences, based on 24-hour averages, ranged from 4 to 10° throughout the season.

The soil temperature treatment differences may be explained by examining the effects of the plastic film. Black plastic film is not transparent to radiation. Therefore, the energy which passes through the film must be absorbed by one surface, conducted through the film, and reradiated at the other surface. When the plastic film is placed on the ground so that the air movement underneath is restricted, a film of moisture condenses on the lower side of the plastic. The condensed moisture layer and the plastic film acting as a greenhouse, effectively trap long wave terrestrial radiation. The color of the plastic film, the "greenhouse effect," and the absence of evaporation from the plastic surface are the causes of the treatment temperature differences.

A review of the literature concerning the effect plastic films have on soil temperatures has revealed some contradictory statements which, for the most part, can be resolved by considering the moisture content of the soil surface. Honma *et al.* (8), reporting mean soil temperatures for 15 sunny days, 3 inches below the surface of exposed and black plastic covered mineral soils, indicated a maximum temperature of 77° in the exposed soil and 76° in the black plastic covered soil. However, the moisture content of the soil was not disclosed. In a study of seed germination, Army and Hudspeth (1) reported soil temperatures and soil moisture content. Their data (Table 1) indicate a treatment temperature difference of 3 to 7°. These temperature differences were inversely related to the treatment soil moisture differences; that is, the smaller temperature difference was associated with the greater moisture difference or the drier check.

Table 1. Average soil temperatures and soil moisture contents for the 0 to  $\frac{1}{4}$ -inch layer. (Data after Army and Hudspeth)

	July 30-Aug. 6		Sept. 24-Oct. 1	
	Plastic <sup>1</sup>	Check	Plastic <sup>1</sup>	Check
Maximum soil temperature	110	107	76	69
Percent moisture content	19.0	3.3	19.0	8.9

<sup>1</sup> Black plastic film

Willis (12) conducted an experiment in which corn was grown through various plastic films 36 inches wide. His data, Table 2, clearly indicate the effect of soil moisture content upon the treatment temperature differences. The soil temperatures under the black polyethylene, at the 4-inch depth, were warmer than the bare soil if the surface of the bare soil was moist. This agrees with the soil temperature data presented in this paper since a high soil moisture content was maintained throughout the experiment. The data of Willis also show the effect of other plastic films. Clear and white plastic film were not used in this experiment; but in 1957, temperatures over 130°F were measured immediately beneath a clear plastic film. These temperatures were fatal to about 50% of the corn seedlings.

Table 2. Average soil temperature, °F, 4-inch depth, 4 pm, as affected by differently colored polyethylene film. (Data after Willis)

Treatment	May				June	July	August	
	10	12	17	31	20	27	8	19
Clear polyethylene film	79.8	73.1	84.9	81.0	86.8	85.6	80.6	86.0
White polyethylene film	68.2	64.7	74.8	72.8	78.6	82.8	78.4	82.9
Black polyethylene film	70.0	65.9	74.6	74.2	82.7	84.1	78.7	84.6
Bare soil	67.2	62.2	75.6	72.3	81.7	84.5	79.3	86.2
Surface soil condition <sup>1</sup>	wet	wet	dry	wet	moist	dry	dry	dry

<sup>1</sup> Estimated by authors from rainfall data

### Air temperatures

The air temperatures at 5 cm (see Figure 3), are more variable than the 6-mm soil temperatures.

The times of occurrence of the daily maximum air temperatures were the same for both treatments, and were similar to the occurrence of the 6-mm soil temperatures (1500 to 1600 in June and 1300 to 1400 in July, August, and September). The 5-cm minimum air temperatures occurred about 0400 hours throughout the season in both treatments.

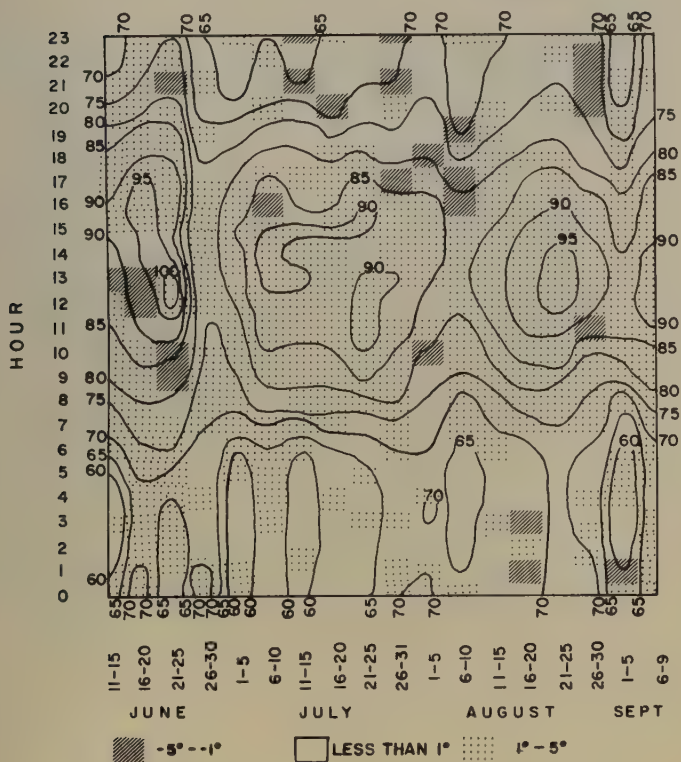


Figure 3. Diurnal and seasonal 5-cm air temperatures for the plastic covered plots and the temperature differences between treatments.

The 5-cm air temperatures in the plastic covered plots were generally warmer, during the daylight hours, than the 5-cm air temperatures in the natural plots (see shaded area, Fig. 3). The greatest treatment differences occurred at midday in late August and early September, and were 3 to 5°. The rest of the temperature differences were 1 to 2°.

The air temperatures at the crop surface height are presented in Fig. 4. The occurrences of the daily maximum and minimum temperatures, at the crop surface height, were similar to the occurrences at the 5-cm height, but were generally cooler than the temperatures at the 5-cm height.

Practically all of the treatment temperature differences (shaded areas, Fig. 4) at the crop surface height, were 1° or less. These differences do not appear to be a direct result of the treatments, but partially rounding differences in computing the 5-day averages.

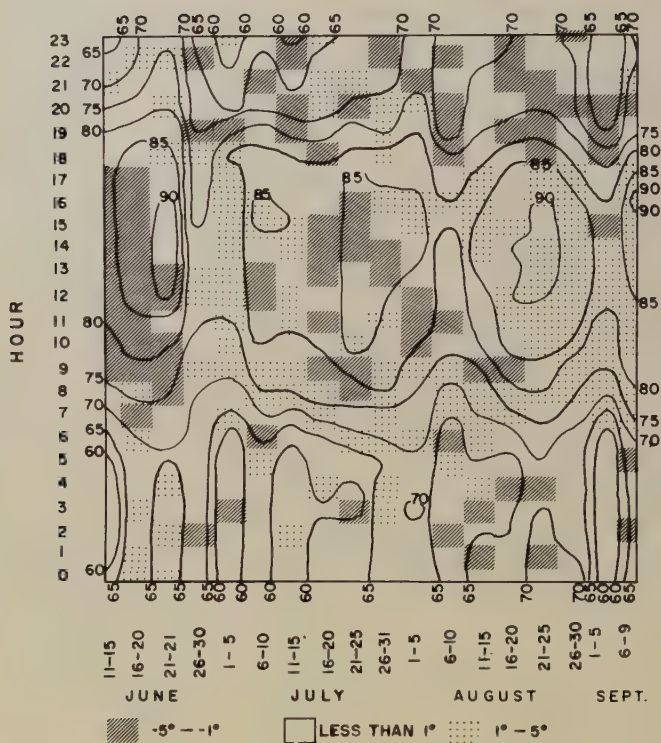


Figure 4. Diurnal and seasonal crop surface temperatures for the plastic covered plots and the temperature difference between treatments.

The air temperatures, in both treatments, at the 30-cm height and the standard screen height (within the crop cover) were similar to the air temperatures at the 5-cm and the crop surface height. Generally, the air temperatures decreased with increasing height.

The effect of the treatments is different for the air and soil temperatures. Generally, the greatest soil temperature treatment differences were observed during the night, while the greatest air temperature treatment differences were observed during the daylight hours. The air temperature treatment differences may be explained by the fact that some of the energy which passes through the plant canopy, in the natural plots, is used in evaporation of soil moisture. This is not the case in the plastic covered plots; more of the energy is used for heating the air. Neither treatment seemed to have much effect on the nighttime temperatures.



The air temperature treatment differences were very small and were usually less than  $1^{\circ}$  when the daily temperature averages were computed. In general, the treatments, although having a large effect on soil temperatures, had very little effect on the air temperatures.

#### Radiation Trends

Solar radiation and net radiation trends are shown in Fig. 5. Solar radiation, as measured by the Eppley pyrheliometer located on top of the Agronomy building at Iowa State University, generally decreased from June 22 to September 10 as the sun moved farther south. The net radiation, as measured by the miniature net radiometers located 1 m above the crop surface, remained nearly uniform. Net radiation, measured

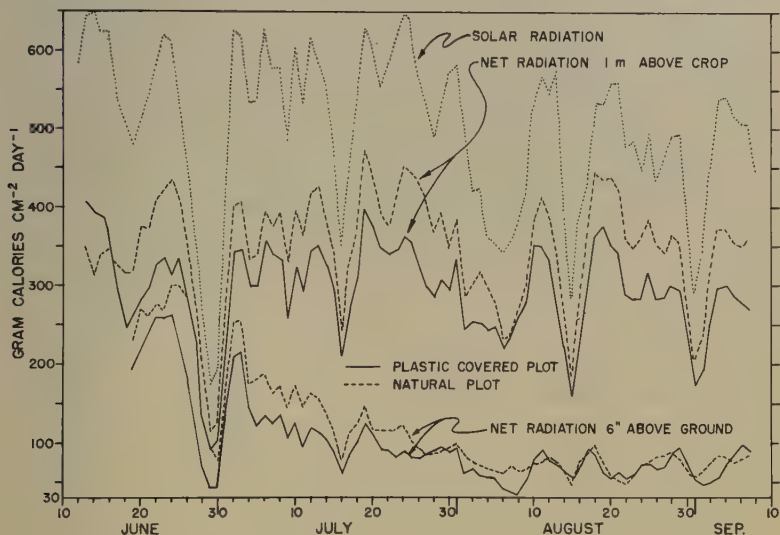


Figure 5. Three-day-running averages of solar radiation and net radiation (1 m above the crop and 6 inches above the soil surface).

6 inches above the soil or plastic surfaces, decreased as the corn grew taller. The peaks on the 6-inch net radiation curve of the natural plots coincides with the times when the soil surface was wet. Less net radiation was measured 1 m above the crop in the plastic covered plots than in the natural plots. This difference in net radiation, measured 1 m above the crop, may be explained by the warmer soil temperatures in the plastic covered plots and by the surface roughness. The warmer soil temperatures and the consequently much warmer plastic temperatures would produce more outgoing radiation, while the smoothness of the plastic may tend to reflect more incoming radiation.

Net radiation measured 1 m above the crop surface will be referred to as plant-soil energy and net radiation measured 6 inches above the ground will be referred to as soil energy. The difference between these, or

$$\text{plant-soil energy} - \text{soil energy} = \text{plant energy}.$$

The soil energy is assumed to be used to heat the soil, to heat the air near the ground, and to evaporate water. The plant energy is assumed to be used in transpiration, photosynthesis, and heating the air.

The natural plots received more plant energy than did the plastic covered plots. This difference in plant energy may be ascribed to the differences in soil temperatures, and soil or plastic roughness. The difference in available plant energy in the two treatments is quite great when integrated over a period of the whole season. The reduced plant energy in the plastic covered plots may decrease the water demands in the plants and thus reduce the stress upon the plants.

The energy balance for both treatments may be altered with different plants, planting rates and soil moisture conditions. Army and Hudspeth (1), working with noncropped 2-foot square plots, reported net radiation of 0.935 langley over bare soil and 1.054 langley over black plastic. These observations were made at 11:30 a.m. on May 29, under a cloudless sky; however, no indication of the moisture content of the soil was given.

## Plant Development

### Root growth

Two root samples were taken from each treatment. The root samples consisted of a north-south section of a hill of corn approximately 3 inches wide, 40 inches long and 36 inches deep. Unfortunately these root samples were not taken until in early December; consequently, most of the finer roots were lost by decomposition or breakage due to freezing and thawing. The corn on the plastic covered plots formed 3 to 4 rings of brace roots, while the corn in the natural plots formed 1 to 2 rings of brace roots. Most of the brace roots, in the plastic covered plots, formed above the plastic and did not penetrate it.

The corn, in the natural plots, apparently developed a better root system, both horizontally and vertically, than the corn in the plastic covered plots. This better root system was developed even though the soil moisture content was greater in the natural plots than in the plastic covered plots. The difference in root development might be explained by examining the effect of temperature upon root development. Dickson (5) working with corn, reported that the largest root systems developed at soil temperatures of about 75°, irrespective of the age of the plant. The average seasonal soil temperature (at 10 cm) was 78.2° in the plastic covered plots and 74.0° in the natural plots. The soil temperatures in the plastic covered plots may have been enough above optimum to hinder root development; however, the soil temperatures in the natural plots apparently were closer to the optimum.

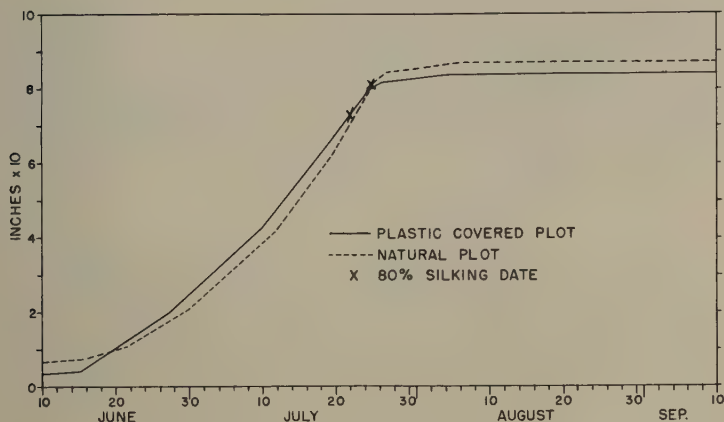


Figure 6. Crop growth.

#### Aerial growth

The plastic films were laid during the period from June 5 to June 9, 1959. The corn on these plots suffered some injury as a result of sealing the plastic around each hill of corn, and required a few days to recover from this injury. After recovery date (i.e. June 15, 1959, Fig. 6), the corn on the plastic covered plots grew more rapidly than did the corn on the natural plots (Fig. 6, June 15 to July 24). After July 24, the corn on the natural plots reached a greater height than the corn in the plastic covered plot. This greater height was stalk elongation. There was no difference in leaf area between the treatments at this stage. The corn on the plastic covered plots was more advanced physiologically than the corn on the natural plots. This advancement was indicated by the 3-day difference in the 80% silking date (Fig. 6), and was also evident in visual appearance at maturity. It is interesting to note that the higher 6-mm soil temperature in the plastic covered plots, from June 5 (placement of the plastic) until July 22 (80% silking date), is equivalent to 3.05 more mean natural plot days, which is the same as the difference in the 80% silking dates. That is, the corn on both treatments required the same number of mean natural plot days to reach 80% silking. Willis (12) also reported earlier emergence, silking, and maturity of corn with increased soil temperatures. Emmert (4) and Carolus and Downes (2) reported earlier maturity of melons, snap beans, tomatoes, and other crops grown through black plastic mulches.

#### Yields

The corn on the plastic covered plots produced more grain and dry matter than the corn on the natural plots. An equivalent of 123.3 bushels of shelled corn and 12,594 pounds of dry matter per acre were harvested in the plastic covered plots, while the natural plots produced 114 bushels of shelled corn and 11,459 pounds of dry matter per acre. Willis (12)

reported increased yields of corn with increased soil temperatures and that the growth of plants and the production of dry matter increased linearly with increasing soil temperatures for the range tested ( $60^{\circ}$  to  $70^{\circ}$ ). He also stated that there were no differences in uptake of the nutrients, nitrogen, potassium or phosphorus, due to treatments. Higher yields on plastic covered plots have been reported by Emmert (4), Carolus and Downes (2), Clarkson and Frazier (3), and others.

## SUMMARY AND CONCLUSIONS

An experiment was conducted at Ames, Iowa, during the summer of 1959 to determine the effect of black plastic film (placed on the soil surface) upon the micro-climate and plant growth. Micro-climate measurements consisted of soil temperatures, air temperatures, and net radiation.

At the depths measured, the soil temperatures in the plastic covered plots were warmer and were subject to less fluctuation than the soil temperatures in the natural plots. The greatest temperature differences occurred at night.

The diurnal soil temperature ranges were greater in the natural plots. The diurnal temperature range in both treatments was nonlinearly correlated with net radiation (measured 6 inches above the soil or plastic surface).

The daily maximum soil temperatures occurred approximately 2 hours earlier in the natural plots. The daily minimum soil temperatures occurred about the same time in both treatments.

The air temperatures were more variable than the soil temperatures and tended to decrease with height. The air temperatures, at the lower levels, in the plastic covered plots tended to be warmer during the daylight hours; but this was less than  $1^{\circ}$  when the 24-hour average was computed.

The daily maximum and minimum air temperatures occurred at the same time in both treatments.

In general, the treatments, although having a large effect on soil temperatures, had very little effect on the air temperatures.

Less net radiation was measured 1 m above the crop and 6 inches above the ground in the plastic covered plots than in the natural plots. The difference between net radiation measured at these two levels (plant energy) was also less in the plastic covered plots. This reduced plant energy may reduce the demand for water by the corn in the plastic covered plots and thereby reduce plant stress.

The corn in the plastic covered plots produced 3 to 4 rings of brace roots, while the corn in the natural plots produced 1 to 2 rings of brace roots. The corn in the natural plots apparently produced a better root system, both horizontally and vertically.

The corn in the plastic covered plots grew more rapidly from June 15 to July 24; however, the corn in the natural plots reached a greater height. There was no difference in leaf area between the treatments at maximum height.

The corn in the plastic covered plots was more advanced physiologically than the corn in the natural plots as indicated by the difference in the 80% silking dates and visual appearance at maturity.

The corn in the plastic covered plots produced more corn and dry matter than the corn in the natural plots.

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# CARBON DIOXIDE FIXATION BY THE GENUS MYCOBACTERIUM<sup>1</sup>

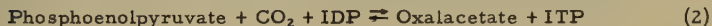
T. Myoda and C.H. Werkman

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of Science and Technology  
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That carbon dioxide may have an influence on the growth of the tubercle bacillus was first indicated by Wherry and Ervin (1918). Historical studies suggest some role for carbon dioxide in the growth of Mycobacterium (Rockwell and Highberger, 1926; Masur, 1926; Davies, 1940; Schaefer et al., 1955). While carbon dioxide tension does influence certain physical factors affecting the cell, such as permeability of membrane, or pH of the medium, these factors are probably of minor importance when compared to the obligatory utilization of carbon dioxide in the metabolism of the organism.

The early studies, however, dealt with bactericidal and inhibitory action. Since 1935 when Wood and Werkman first proposed the concept of carbon dioxide utilization in heterotrophic metabolism, the effect of carbon dioxide has been studied in connection with the metabolism.

The Wood-Werkman reaction was intended to represent an over-all reaction and details were expected to be added. Kalnitsky and Werkman (1944) while considering the energy requirements of the reaction postulated that phosphorylation of intermediates would be shown to occur. It has become clear that phosphoenolpyruvate is the carbon dioxide acceptor in two distinct reactions for the fixation of carbon dioxide to form oxalacetate. One is catalyzed by phosphoenolpyruvic carboxylase (Bandurski and Greiner, 1953), and the other is catalyzed by oxalacetic carboxylase (Utter and Kurahashi, 1953). These enzymes catalyze the following reactions respectively:



The present investigation was undertaken to determine the presence and function of these enzyme systems in an acid-fast organism, Mycobacterium phlei.

<sup>1</sup> Supported in part by a grant of the Department of Health, Education, and Welfare, Public Health Service.

## METHODS AND MATERIALS

Reagents

All reagents were commercial preparations: Oxalacetic acid (OAA) and phosphoenolpyruvate (tricyclohexylamine salt) (PEP), California Foundation for Biochemical Research; sodium inosine di- and triphosphate (IDP and ITP), sodium adenosine di- and triphosphate (ADP and ATP), sodium uridine di- and triphosphate (UDP and UTP), sodium cytidine di- and triphosphate (CDP and CTP), sodium guanosine di- and triphosphate (GDP and GTP), Pabst Laboratories;  $\text{BaC}^{14}\text{O}_3$ , Oak Ridge National Laboratory; reduced glutathione (GSH), Nutritional Biochemicals Corporation.

Preparation of Cell-Free Extracts

Mycobacterium phlei ATCC 10142 was grown in a modified Dubos' medium which contained 1% glucose and no  $\text{CuSO}_4$  for 48 hours at  $37^\circ\text{C}$ . The culture was vigorously aerated by a stream of air sterilized by passage through a sterile cotton filter. The cells were harvested by centrifugation, washed twice with distilled water, and 5 grams, wet weight, of the cells were suspended in 35 ml of 0.2 M tris buffer. Then a small amount of Alcoa Alumina A 301 was added. The suspension thus obtained was subjected to sonic treatment in a Raytheon 9 kc. sonic oscillator at  $4^\circ\text{C}$  for 25 minutes. Debris was removed by centrifugation at 18,000 rpm for 25 minutes in an International refrigerated centrifuge. The cell-free extracts thus obtained were subjected to dialysis. Dialysis was carried out in a cellophane bag against 500 ml of 0.002 M tris buffer of pH 7.4. The buffer was stirred constantly by a magnetic stirrer and frequently changed. In a previous report (Baugh et al., 1959) an absolute requirement for a divalent metal ion for fixation was demonstrated. An absolute requirement for a nucleotide for the exchange reaction between oxalacetate and  $\text{NaHC}^{14}\text{O}_3$  was demonstrated. Therefore the dialysis was continued until the extract showed no fixation without an additional metal ion and no exchange reaction without an addition of a nucleotide. This dialysis usually takes at least 60 hours. The expression "no endogenous" in the following tables or figures indicates no divalent metal ions, no nucleotides, and no residual substrate in the extract employed. The cell-free extracts were kept frozen ( $-20^\circ\text{C}$ ).

Preparation of  $\text{NaHC}^{14}\text{O}_3$ 

Radioactive bicarbonate was obtained by trapping  $\text{C}^{14}\text{O}_2$  (liberated from  $\text{BaC}^{14}\text{O}_3$  by 10% perchloric acid) in 0.05 NaOH solution in an evacuated system.

Assay of radioactivity

Radioactivity was measured with a lead-shielded end-window (mica) Geiger-Müller tube (Tracerlab type TGC2) having a window thickness of 1.8 mg per  $\text{cm}^2$ . Counts were corrected for background and expressed as counts per minute (c/m). The counting time was selected so as to make the standard deviation within 5% of the net count.

In a previous report (Baugh et al., 1959) it was found that the final product of the fixation was oxalacetate having the radioactive carbon in

its  $\beta$ -carboxyl group. Therefore in this investigation an aliquot of the reaction mixture was placed on a glass planchet and the activity fixed was counted.

Kodak No-screen medical X-ray film was used to locate radioactive compounds isolated by paper chromatography and paper electrophoresis.

#### Determination of Protein

The biuret method of Gornall *et al.* (1949) was used to determine the protein content of the cell-free extracts. An interference of tris buffer was minimized by the analysis of the separated protein precipitate as described by Robinson and Hogden (1940).

### EXPERIMENTAL

Reactions were carried out in Warburg flasks with two side arms, under an atmosphere of nitrogen. The main chamber contained the substrate, buffer, and any additional cofactors or nucleotides. In case of the exchange reaction, the substrate (oxalacetate) was usually placed in a separate side arm because of its instability. The contents of the side arms were tipped into the main chamber after replacement of the gas phase and temperature equilibration at 31°C. The reactions were stopped after a selected period of time by the addition of trichloroacetic acid. Proteins precipitated were centrifuged down. An aliquot of supernatant was placed on a glass planchet for the determination of radioactivity fixed.

Liberation of inorganic phosphate. Although an attempt has been made to determine the liberation of inorganic phosphate (reaction 1) it could not be shown because of the minute amount produced.

Requirement of divalent metal ion. In a previous paper (Baugh *et al.*, 1959) an absolute requirement for divalent metal ion was demonstrated. It was found that  $Mg^{++}$ ,  $Mn^{++}$ , or  $Co^{++}$  can fulfill the requirement.

Specificity of nucleotides. The reversible fixation of carbon dioxide (reaction 2) requires inosine polyphosphate as a phosphate acceptor or donor. Attempts were made to determine the effect of other nucleotides on the fixation. The results are summarized in Tables 1 and 2.

As shown in Tables 1 and 2 inosine- and guanosine-polyphosphate have a great effect on the fixation and the exchange reaction, especially the latter compound. The effect of the other nucleotides seems to vary with metal ions present and with extracts. In some cases the rate of the exchange reaction was greater with ATP than with ITP or GTP. The reason is not known. Results of the nucleotide-independent fixation with magnesium ions (Table 1, No. 7) are not in agreement with the results reported previously (Baugh *et al.*, 1959). The difference is thought to be due to the freshness of the extracts. The fresh extracts may fix carbon dioxide in the presence of magnesium ions. Although the crude extracts kept the ability to fix carbon dioxide for some time (a few months), the manganese-fractionated extracts and well-dialyzed extracts gradually lose their enzymatic activity. It is also noticed in some cases that the addition of nucleotide does not increase the rate of fixation even with freshly prepared extracts, unless reduced glutathione (GSH) is added.

Table 1. Effect of various nucleotides on the fixation<sup>a</sup>.

The reactions were carried out at 31°C for an hour. Two-tenths ml of 50% trichloroacetic acid (TCA) was added to the reaction mixture to stop the reaction. Gas phase: 100% N<sub>2</sub>.

No.	Ion	Nucleotide	Activity fixed (c/m/100 $\mu$ l.)	
1	MnCl <sub>2</sub>	None	376 <sup>b</sup>	620 <sup>c</sup>
2	"	IDP	894	1244
3	"	UDP	248	566
4	"	CDP	224	392
5	"	ADP	232	226
6	"	GDP	1104	2438
7	MgCl <sub>2</sub>	None	246	44
8	"	IDP	528	156
9	"	UDP	365	146
10	"	CDP	40	52
11	"	ADP	444	52
12	"	GDP	682	264
13	CoCl <sub>2</sub>	None	196	284
14	"	IDP	768	822
15	"	UDP	574	718
16	"	CDP	288	426
17	"	ADP	366	418
18	"	GDP	1094	1540

<sup>a</sup>The basal system contained 100  $\mu$ moles of tris buffer (pH 7.4), 2  $\mu$ moles of PEP and 2.5  $\mu$ moles of NaHC<sup>14</sup>O<sub>3</sub> ( $1 \times 10^7$  c/m). Two  $\mu$ moles of nucleotide were used. The total volume was 2.0 ml. Five  $\mu$ moles of Mg<sup>++</sup> or Mn<sup>++</sup> and 4  $\mu$ moles of Co<sup>++</sup> were used, respectively.

<sup>b</sup>The results obtained from the addition of 0.3 ml of 64-hour dialyzed extracts having no endogenous metabolism.

<sup>c</sup>The results obtained from the addition of 0.3 ml of 67-hour dialyzed extracts (2.55 mg protein per ml) containing no endogenous metabolism.



Table 2. Effect of various nucleotides on the exchange reaction<sup>a</sup>.

No.	Ion	Nucleotide	Activity fixed (c/m/100 $\mu$ l.)
1	MnCl <sub>2</sub>	None	0
2	"	ITP	316
3	"	UTP	214
4	"	CTP	82
5	"	ATP	268
6	"	GTP	1108
7	MgCl <sub>2</sub>	None	0
8	"	ITP	758
9	"	UTP	136
10	"	CTP	32
11	"	ATP	214
12	"	GTP	1168
13	CoCl <sub>2</sub>	None	0
14	"	ITP	18
15	"	UTP	0
16	"	CTP	14
17	"	ATP	34
18	"	GTP	88

<sup>a</sup>The basal system was the same as in Table 1, except 3  $\mu$ moles of OAA instead of PEP were used. The extracts and ions were placed in a side arm together. Two  $\mu$ moles of a nucleotide were used. The extracts used were 0.3 ml of 67-hour dialyzed extract containing no endogenous and 2.55 mg protein content per ml. The other conditions were the same as in Table 1.

This anomaly is thought to be due to the variation of efficiency of a Raytheon sonic oscillator and to dialysis.

Effect of glutathione. Since the addition of reduced glutathione, in some cases, stimulates the fixation of carbon dioxide, the effect of glutathione was determined. The results with 5-week old extracts are shown in Table 3.

The stimulating effect of reduced glutathione was significant with the magnesium ion. Reduced glutathione appears to be required by the nucleotide-independent system with magnesium ion. The oxidized glutathione did not stimulate the reactions. In the previous report (Baugh *et al.*, 1959) it was concluded that magnesium ions cannot be substituted for manganese ions in the nucleotide-independent fixation, this could be due to the absence of reduced glutathione in the extracts. With either cobalt or manganese ions the addition of glutathione slightly inhibits the reactions.

Table 4 shows the results with the extracts which have been kept in a freezer for one month and then dialyzed for 70 hours.

The optimal concentration of glutathione varies with the extract and length of storage.

Table 3. The effect of reduced glutathione on the fixation<sup>a</sup>.

No.	Ion	Addition	Activity fixed (c/m/100 $\mu$ l.)
1	MgCl <sub>2</sub>	None	40
2	"	GSH	388
3	"	GSH, IDP	1142
4	"	IDP	918
5	CoCl <sub>2</sub>	None	252
6	"	GSH	230
7	"	GSH, IDP	1426
8	"	IDP	1742
9	MnCl <sub>2</sub>	None	360
10	"	GSH	354
11	"	GSH, IDP	1518
12	"	IDP	1738

<sup>a</sup>The basal system contained 100  $\mu$ moles or tris buffer (pH 7.4), 3  $\mu$ moles of PEP, and 0.3 ml of 64-hour dialyzed extracts. GSH added was 5  $\mu$ moles. IDP: 3  $\mu$ moles. The other conditions were the same as in Table 1.

Table 4. Effect of reduced glutathione on the fixation and the exchange reactions<sup>a</sup>.

No.	Ion	Substrate	Addition	Activity fixed (c/m/100 $\mu$ l.)
1	MnCl <sub>2</sub>	PEP	None	240
2	"	"	GSH	228
3	"	"	GSH, IDP	1014
4	"	"	IDP	230
5	MgCl <sub>2</sub>	"	None	0
6	"	"	GSH	124
7	"	"	GSH, IDP	638
8	"	"	IDP	0
9	MnCl <sub>2</sub>	OAA	ITP	570
10	"	"	ITP, GSH	80
11	MgCl <sub>2</sub>	"	ITP	182
12	"	"	ITP, GSH	274

<sup>a</sup>Two tenths of a ml of the extracts (3.2 mg protein/ml) were used. The other conditions were the same as in Table 3.

Optimal concentration of substrate. As shown in Figure 1, the fixation is proportional to the concentration of the substrate within a certain range. The rate of the fixation is doubled when the amount of the extract is doubled. When the extract was boiled for two minutes in a water bath the activity was lost completely.

Optimal concentration of nucleotide. As shown in Figure 2, maximal fixation occurs when the ratio of phosphoenolpyruvate and nucleotide is equimolar. The rate of fixation fell off sharply when this ratio was less than one and more slowly when greater than one.

Optimal concentration of  $\text{NaHC}^{14}\text{O}_3$ . Typical results are illustrated in Figure 3. Since the two enzyme systems have not been separated yet, the optimal concentration of  $\text{NaHC}^{14}\text{O}_3$  for the nucleotide-dependent system was plotted by subtracting the value of the nucleotide-independent fixation (Figure 3, C). Under the experimental conditions employed the maximal rate of fixation will be obtained with about 10  $\mu\text{moles}$  of  $\text{NaHC}^{14}\text{O}_3$ .

Optimal duration of the reaction. The relationship between the fixation and the reaction period was determined. Under the conditions as shown in Figure 4, the reaction reaches its maximal point after 2 hours.

Optimal pH. The pH optima for the fixation reactions, i. e. nucleotide-independent and dependent and the exchange reaction are shown in Figures 5 and 6. It is of interest that the rate of fixation is very low when phosphate buffer is used. According to Tchen and Vennesland (1955) the rate of oxalacetate formation is stimulated by inorganic phosphate even though the stimulation is not completely reproducible quantitatively. The original phosphoenolpyruvic carboxylase has a broad pH maximum over the interval pH 7.5 to 9.5 (Bandurski, 1955). The decarboxylation of oxalacetate in the presence of oxalacetic carboxylase is maximum at pH 6.4 (Utter et al., 1954). The rate of fixation was also low with glycylglycine buffer. The glycylglycine buffer stimulates greatly the nonenzymatic decarboxylation of oxalacetic acid (Utter et al., 1954).

Specificity of substrate. With either malate or pyruvate as a substrate the fixation could not be demonstrated in the presence of manganese, magnesium or cobalt ions. The spectrophotometric determination also did not show the presence of malic dehydrogenase in the well-dialyzed extract.

## DISCUSSION

In a previous paper (Baugh et al., 1959) evidence is given for the presence of (at least) two different reactions for the fixation of carbon dioxide into oxalacetate in cell-free extracts of *M. phlei*. One is an irreversible reaction similar to the one catalyzed by the enzyme phosphoenolpyruvate carboxylase and the other is a reversible nucleotide-dependent reaction similar to the reaction catalyzed by the enzyme oxalacetic carboxylase.

The present investigation was undertaken to determine more detailed properties of the reactions.

The original phosphoenolpyruvic carboxylase obtained from spinach leaves requires magnesium ion and a reducing agent (Bandurski and Greiner, 1953), whereas that from wheat germ does not show the absolute

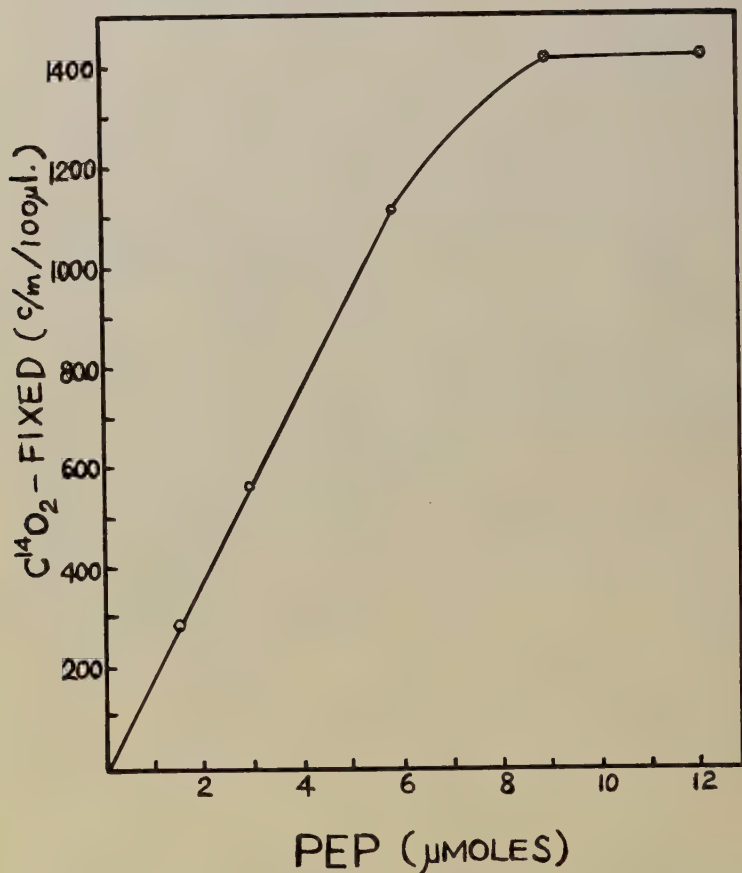


Figure 1. Optimal concentration of substrate.

The basal system contained 100  $\mu$ moles of tris buffer (pH 7.4), 2.5  $\mu$ moles of  $NaHC^{14}O_3$  ( $1 \times 10^7$  c/m), 3  $\mu$ moles of IDP, 5  $\mu$ moles of  $MnCl_2$ , and 0.1 ml of 67-hour dialyzed extracts containing no endogenous. The protein content was 2.55 mg per ml. The concentration of PEP was varied. The other conditions were the same as in Table 1.

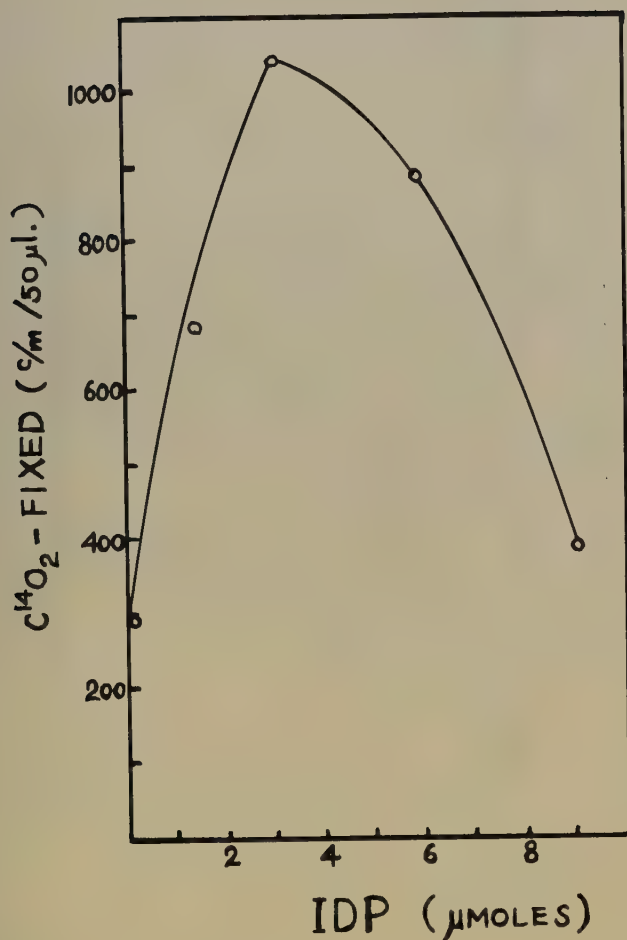


Figure 2. Optimal concentration of nucleotide.

3/10 ml of 67-hour dialyzed extracts (2.55 mg protein/ml) were used. The other conditions were the same as in Figure 1 except the PEP used was fixed to 3 μmoles and the concentration of IDP was varied.



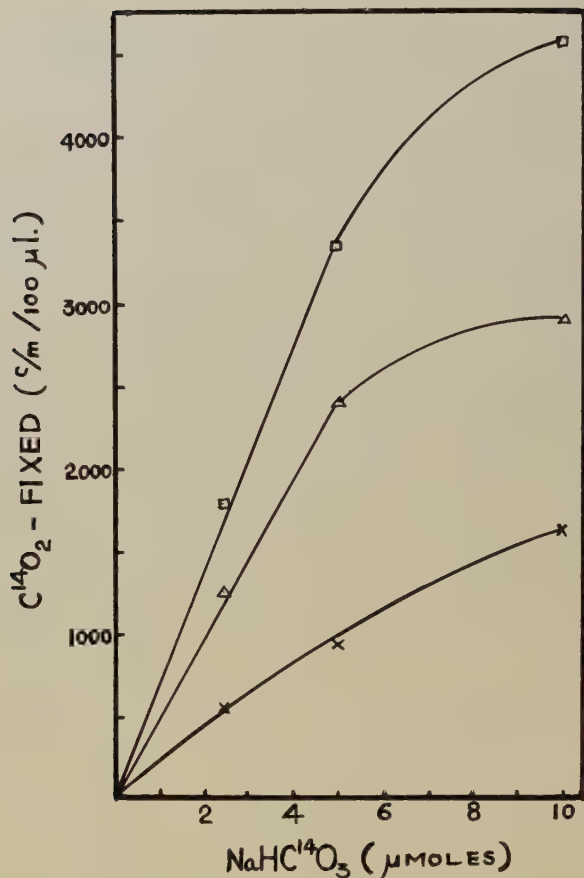


Figure 3. Optimal concentration of  $\text{NaHC}^{14}\text{O}_3$ .

The basal system contained 100  $\mu\text{moles}$  of tris buffer (pH 7.4), 5  $\mu\text{moles}$  of  $\text{MnCl}_2$ , 3  $\mu\text{moles}$  of PEP, and 0.3 ml of 67-hour dialyzed extracts (2.55 mg protein / ml). The concentration of  $\text{NaHC}^{14}\text{O}_3$  was varied.

- - □ (A): containing 3  $\mu\text{moles}$  of IDP
- x - x (B): without IDP
- Δ - Δ (C): (A) - (B)

The other conditions were the same as in Figure 1.

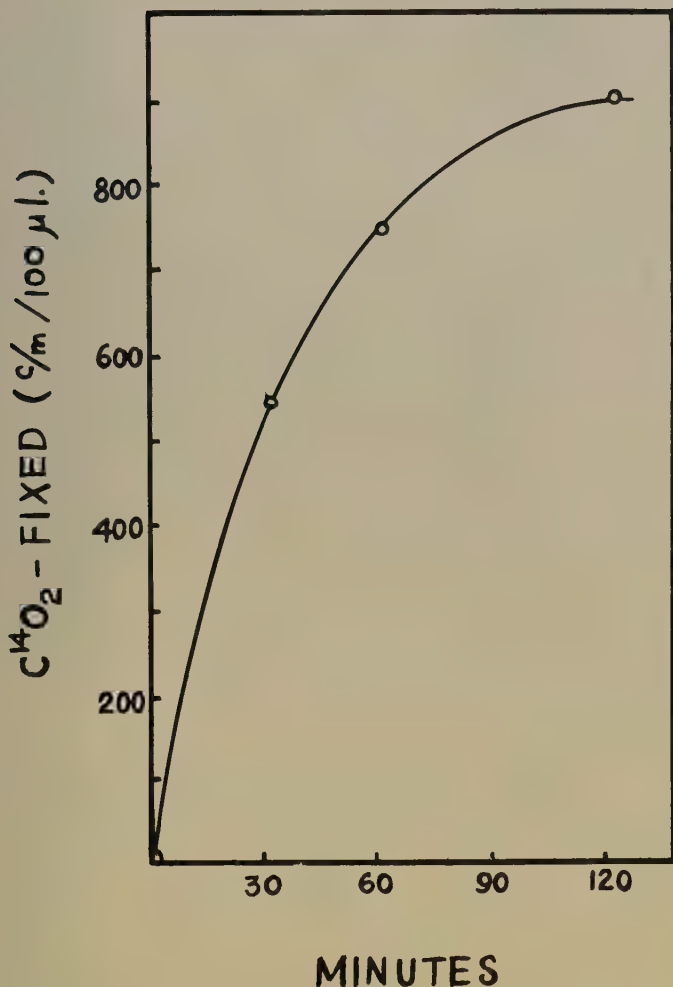


Figure 4. The relationship between the fixation and reaction period.

The system contained 100  $\mu$ moles of tris buffer (pH 7.4), 6  $\mu$ moles of PEP, 5  $\mu$ moles of  $MnCl_2$ , 5  $\mu$ moles of  $NaHC^{14}O_3$  ( $2 \times 10^7$  c/m), and 0.2 ml of 67-hour dialyzed extracts. The reaction was stopped at indicated interval.

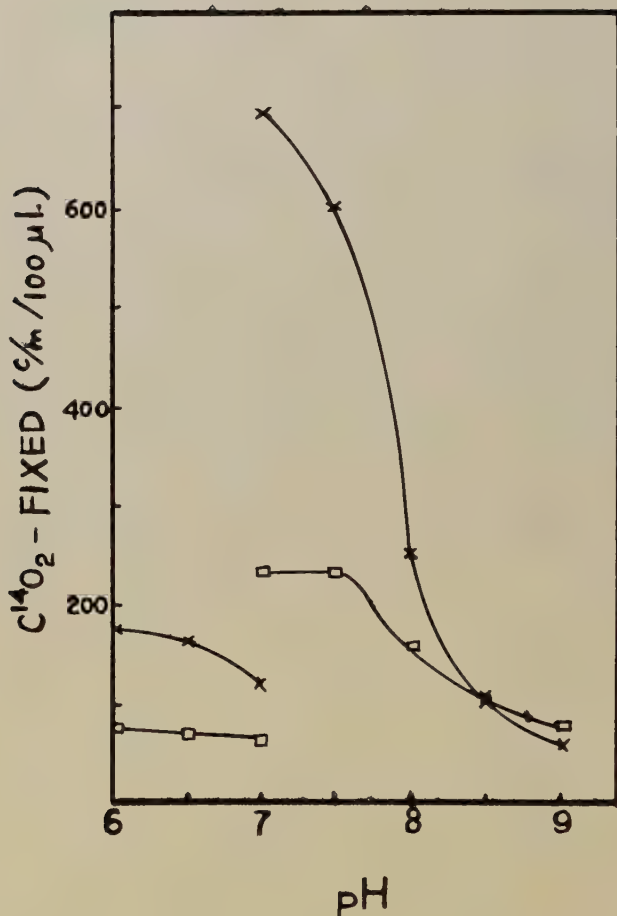


Figure 5. pH optimum for nucleotide-independent and dependent fixations.

Seventy  $\mu$ moles of phosphate buffer were used for a pH range from 6.0 to 7.0. Tris buffer (70  $\mu$ moles) was used between pH 7.0 and 9.0.

x - x: Nucleotide-dependent system (containing 3  $\mu$ moles of IDP)

□ - □: Nucleotide-independent system.

Two-tenths ml of 67-hour dialyzed extracts were used.

The other conditions were the same as in Figure 1.

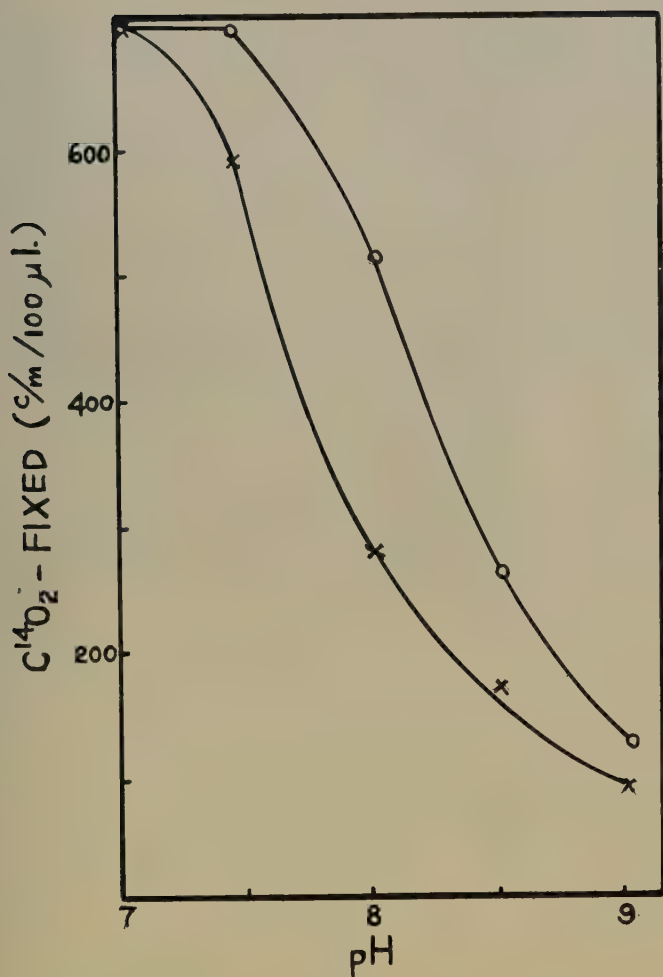


Figure 6. pH optimum for the exchange reaction.

The experimental conditions were the same as in Figure 5, except 3  $\mu$ moles of OAA instead of PEP and ions indicated below.

x - x: 5  $\mu$ moles of  $MnCl_2$

o - o: 5  $\mu$ moles of  $MgCl_2$

requirement for magnesium ions and no requirement for the reducing agent is shown (Tchen and Vennesland, 1955). In the latter case manganese can replace magnesium for the maximum activity. Although no absolute requirement for either manganese or magnesium was shown even after dialysis, the addition of EDTA (ethylenediamine tetraacetic acid) inhibited the reaction completely in the absence of added ion. This inhibition can be reversed by adding an excess of magnesium or manganese ions indicating the necessity of a divalent metal ion. They used not only the isotopic method but the spectrophotometric method. With the extract of *M. phlei* it is impossible to determine the mechanism by any other method than the isotopic method because of the small amount of fixation. The present study showed that the effect of the divalent metal ion varies with the freshness of the extract and with the length of dialysis. Although it was thought that magnesium was inactive on the nucleotide-independent system (Baugh et al., 1959), the addition of reduced glutathione promoted the reaction. These results apparently show that the oxidation-reduction potential of the extract affects the fixation. The present study indicates that  $Mn^{++}$ ,  $Mg^{++}$ ,  $Co^{++}$  can fulfill the requirement of carboxylating enzyme systems.

The effect of reduced glutathione is significant when magnesium is used.

Since an addition of inosine diphosphate stimulated the rate of reaction, the effect of other nucleotides was determined. Since the nucleotide-dependent system is reversible, experiments were devised in two ways. One system contained phosphoenolpyruvate and a nucleoside diphosphate, and the other contained oxalacetate and a nucleoside triphosphate. In both cases inosine and guanosine polyphosphates, especially the latter, were found to be most active. According to Kurahashi et al. (1957) the oxalacetic carboxylase reaction is specific for these two compounds, with the latter more active. The stimulation of fixation by the other nucleotides is due to contamination of these compounds in commercial products and to the presence of nucleoside diphosphokinase in the preparation. On the other hand McManus (1951), with lysed suspensions and cell-free extracts of *Micrococcus lysodeikticus*, could not demonstrate a significant effect of nucleotide, ATP, on the oxalacetic carboxylase reaction. More recently Cannata and Stoppani (1959) reported an enzyme of bakers' yeast which is similar to oxalacetic carboxylase except its nucleotide specificity. This enzyme reaction is only specific for adenosine polyphosphate.

In the case of the extract of *Mycobacterium phlei* the rate of stimulation of other nucleotides varied with the metal ion present and with the extract. The inconsistency is thought to be due to inconsistencies of a Raytheon sonic oscillator and of dialysis. Protein contents of extracts varied from sample to sample. Although several attempts have been made to demonstrate nucleoside diphosphokinase in the extract, conclusive evidence has not been obtained.

The optimal concentration of inosine diphosphate was determined. The rate of fixation was maximum when the concentration of the nucleotide and that of the substrate, phosphoenolpyruvate, were equimolar.

The optimal pH for a nucleotide-independent system was found to be between pH 7.0 and 7.5. The optimal pH for the nucleotide-dependent



system was pH 7 in the presence of both manganese and magnesium. The rate of decomposition of oxalacetate will vary with added compounds. Oxalacetate should be separated from any other component until the reaction is started. In this respect it is desirable to use a Warburg flask with more than two side arms. The rate of fixation is higher in tris buffer than any other buffer used.

In results not shown the optimal concentration of added  $Mn^{++}$  ions under the experimental conditions was about 6  $\mu$ moles. According to Bandurski (1955) one mole of magnesium ions is required for maximal activity. Higher concentrations of the ion may cause inhibition.

The reactions were carried out under an atmosphere of nitrogen. The main reason for this condition is to inhibit the operation of the Krebs' citric acid cycle which might occur after oxalacetate is formed. With well-dialyzed extracts containing no endogenous fixation, however, the rate of fixation did not change even when the reaction was carried out under an atmosphere of air.

### SUMMARY

An extract of Mycobacterium phlei possesses at least two carboxylating systems which catalyze the reaction between phosphoenolpyruvate and carbon dioxide. One is a nucleotide-independent system similar to phosphoenolpyruvic carboxylase and is irreversible. The other system is a nucleotide-dependent system similar to oxalacetic carboxylase and is reversible.

For both reactions a divalent metal ion is required. Magnesium, manganese or cobalt ions fulfill the requirement.

The rate of fixation of carbon dioxide by a nucleotide-dependent system is greater with guanosine and inosine polyphosphate, especially the former. The optimal concentration of a nucleotide is found to be equimolar with the substrate, phosphoenolpyruvate. The effect of other nucleotides such as adenosine, cytidine, and uridine polyphosphates, varies with the divalent ion present and with the preparation.

The effect of reduced glutathione is significant when magnesium ions are used. The optimal concentration of added glutathione varies with freshness of the extract and with storage.

The optimal pH for the nucleotide-independent system is pH 7 to 7.5 in tris buffer. The optimal pH for the nucleotide-dependent system is pH 7.0 in tris buffer in the presence of manganese ion and pH 7.0 to 7.5 in the presence of magnesium. The rate of each reaction is greater in tris buffer than any other buffer used.

The optimal conditions for these fixation systems were investigated.

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EVIDENCE FOR A TRICARBOXYLIC ACID CYCLE  
IN BREVIBACTERIUM LEUCINOPHAGUM<sup>1</sup>

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ABSTRACT

Evidence for the presence and operation of a tricarboxylic acid cycle in Brevibacterium leucinophagum is presented. This consists of (a) growth in the presence of TCA cycle compounds and close relatives as sole sources of carbon; (b) stimulation of oxygen uptake by whole cells by TCA cycle compounds; (c) sensitivity to known inhibitors of TCA cycle reactions; and (d) conversion and accumulation of compounds known to be intermediates of the TCA cycle.

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The functions of the tricarboxylic acid cycle are twofold: to provide energy to drive synthetic reactions by the oxidation of intermediates of carbohydrate dissimilation, and to furnish a supply of intermediates for the synthesis of other compounds (1).

Moses (2) has stated that the only valid evidence for the operation of a TCA cycle is the measurement of flow rates and recycling of compounds through the use of labelled substrates according to the method of Roberts et al. (3). Suggestive evidence for the presence of the TCA cycle has been obtained by many other workers, however, by four general methods: (a) growth in the presence of TCA cycle compounds and close relatives as the sole C source; (b) stimulation of oxidative activity by TCA cycle intermediates; (c) sensitivity to compounds known to inhibit TCA cycle reactions; and (d) chemical conversion and accumulation of compounds known to be intermediates of the TCA cycle.

Kinney and Werkman (4) have identified a number of amino acids formed during the degradation of leucine by Brevibacterium leucinophagum (5). Among these were aspartic and glutamic acids, threonine and isoleucine. They suggested these might be formed from leucine-derived C<sub>2</sub> or C<sub>3</sub> fragments metabolized via the TCA cycle. There is ample evidence that the members of the "aspartic-glutamic families" of amino acids are synthesized in this way by other organisms (6, 7).

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This paper provides evidence for the existence and functioning of a TCA cycle in B. leucinophagum, and thus accounts for the synthesis of these amino acids from  $C_2$  or  $C_3$  intermediates. The occurrence of a TCA cycle has not been reported previously in this genus.

## MATERIALS AND METHODS

The test organism was grown and the cells were harvested as described elsewhere (4). Acetone-dried cells were prepared according to standard techniques.

All chemical compounds used were of reagent grade purity, and were obtained from commercial sources with the exception of fluoroacetate. Sodium fluoroacetate (99% pure) was obtained from the University of Chicago Toxicity Laboratory, 927 East 57th Street, Chicago 37, Illinois.

Growth curves were determined by measurement of the transmittance at 525 m $\mu$  in a "Spectronic 20" colorimeter.

Conventional manometric techniques were used.

Compounds in reaction mixtures of inhibitor experiments were identified chromatographically as previously described (4).

## RESULTS

Previous experiments had shown that B. leucinophagum grows readily in Koser's medium (citrate as sole C source,  $NH_4^+$  as sole N source). Tables I and II show, furthermore, that rapid, abundant growth resulted when known intermediates of the TCA cycle served as sole C source in the presence of  $NH_4^+$ , or when amino acids closely related to TCA cycle compounds served as sole C and N sources.

Table III shows that the oxygen uptake by whole cell suspensions is stimulated by acetate and other members of the TCA cycle ( $\alpha$ -ketoglutarate, malate, citrate, fumarate and succinate).

Reduced oxygen uptake due to the competitive inhibition of succinic dehydrogenase by malonate could not be shown with whole cell suspensions in the presence of succinate or other TCA cycle compounds. On the contrary, slight increases in oxygen uptake due to malonate were noted after lag periods of 40-50 minutes. Moses (8) and other workers have suggested that this ineffectiveness of malonate with whole cells might be due to permeability effects since it can sometimes be overcome by altering the pH of the test system or by the use of esters of malonic acid (9). No differences were noted in experiments with B. leucinophagum at various pH values over the range pH 7-9 in tris buffers or over the range pH 6-8 in phosphate buffers. Whole cells were completely inactive on a variety of substrates over the range pH 4-5 in phthalate buffers.

Acetone-dried cells showed no oxidative activity on malonate, however, and Table IV shows that oxygen uptake on acetate, succinate, fumarate and malate in this system was strongly inhibited by malonate. The competitive nature of this inhibition is demonstrated by its reversal by increasing concentrations of the substrate in the fumarate-malonate system, as shown in Table V.

Table I. Growth of B. leucinophagum on TCA cycle intermediates as sole carbon source.

Age hrs.	O.D. at 525 m $\mu$			
	AC	SUC	AKG	MAL
0	0.02	0.03	0.02	0.02
4	0.04	0.03	0.04	0.04
8	0.09	0.10	0.11	0.04
12	0.17	--	0.28	0.04
16	0.27	--	0.44	--
20	0.45	--	--	0.09
24	0.68	0.27	--	0.12
28	0.82	0.35	0.82	0.18
32	0.95	0.45	0.87	0.22
36	--	0.52	0.90	0.28

Growth medium contained salts, 0.1%  $\text{NH}_4\text{H}_2\text{PO}_4$ , and 0.3% TCA cycle intermediates at pH 7.0. Inoculated with one drop washed 24 hr. cells. Incubated at 30°C.

AC = acetate; SUC = succinate; AKG =  $\alpha$ -ketoglutarate; MAL = malate.

Table II. Growth of B. leucinophagum on amino acids closely related to TCA cycle intermediates.

Age hrs.	O.D. at 525 m $\mu$		
	GLU	ASP	ALA
0	0.03	0.02	0.02
4	0.06	0.10	0.06
8	0.19	0.24	0.15
12	0.35	0.45	0.22
16	0.55	0.63	0.30
20	0.62	0.72	0.41
24	0.70	0.82	0.48
28	0.80	0.89	0.53
32	0.89	0.94	0.57
36	0.92	0.97	0.63

Growth medium contained salts, 0.3% of the amino acid indicated at pH 7.0. Inoculated with one drop washed 24 hr. cells. Incubated at 30°C.

GLU = glutamate; ASP = aspartate; ALA = alanine.



Table III. Oxygen uptake by whole cells of B. leucinophagum on TCA cycle compounds.

Substrate	$\mu\text{l. O}_2$ uptake/hr.
Malate	230
$\alpha$ -ketoglutarate	121
Citrate	165
Fumarate	208
Succinate	313
Acetate	301

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; 5  $\mu\text{M}$  substrate; and 150 mg wet cells in a total volume of 2.0 ml. Center well contained 0.1 ml 20% KOH and a fluted filter paper. Shaken under air at 30°C. Data corrected for endogenous respiration (34  $\mu\text{l. O}_2/\text{hr.}$ ).

Table IV. Effect of malonate on oxidation of TCA cycle compounds by acetone powders of B. leucinophagum.

Substrate	$\mu\text{l. O}_2/\text{hr.}$		% Inhibition
	w/o malonate	w/malonate	
Acetate	378	108	71.4
Succinate	200	11	94.5
Fumarate	259	37	85.7
Malate	232	5	97.8
Malonate	---	4	--

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; 10  $\mu\text{M}$  substrate; 0.3 ml acetone powder suspension (equivalent to 500 mg wet cells) in a total volume of 2.0 ml. Center well contained alkali and a fluted filter paper. Shaken under air at 30°C. Data corrected for endogenous respiration (35-49  $\mu\text{l. O}_2/\text{hr.}$ ).

Table V. Reversal of malonate inhibition of fumarate oxidation by acetone powders of B. leucinophagum.

Fumarate $\mu\text{M}$	Malonate $\mu\text{M}$	$\mu\text{l. O}_2$ uptake per 60 min.	% Inhibition
2.0	---	257	(control)
2.0	2.0	49	81.0
10.0	2.0	115	55.3
20.0	2.0	192	25.3
30.0	2.0	219	14.8
40.0	2.0	227	11.7

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; concentrations of substrates shown; and 0.4 ml acetone powder suspension (equivalent to 700 mg wet cells) in a total volume of 2.0 ml. Center well contained a fluted filter paper + 0.1 ml 20% KOH. Shaken under air at 30°C. Data corrected for endogenous respiration.

Table VI. Effect of chloramphenicol on malonate oxidation by normal and malonate-grown cells of B. leucinophagum.

Variable	$\mu\text{l. O}_2/\text{hr.}$	% Inhibition
Normal cells + malonate	67	68.6
Normal cells + malonate + CMP	21	
Malonate-grown cells + malonate	198	21.2
Malonate-grown cells + malonate + CMP	156	

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; 10  $\mu\text{M}$  malonate; and chloramphenicol (200  $\mu\text{g}/\text{ml}$ ) where indicated. Total volume = 2.0 ml; gas phase = air; temperature = 30°C. Data corrected for endogenous respiration (normal cells = 54  $\mu\text{l. O}_2/\text{hr.}$ ; malonate-grown cells = 39  $\mu\text{l. O}_2/\text{hr.}$ ).

CMP = chloramphenicol.

Table VII. Effect of arsenite on oxidation of TCA intermediates by acetone powders of B. leucinophagum.

Substrate	$\mu\text{l. O}_2/\text{hr.}$	% Inhibition
Acetate	471	77.1
Acetate + arsenite	108	
Succinate	319	86.8
Succinate + arsenite	42	
Malate	267	83.9
Malate + arsenite	43	

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; 10  $\mu\text{M}$  substrate; 10  $\mu\text{M}$  arsenite where indicated; and 0.3 ml acetone powder suspension (equivalent to 500 mg wet cells) in a total volume of 2.0 ml. Center well contained a filter paper + 0.1 ml 20% KOH. Shaken under air at 30°C. Data corrected for endogenous respiration (38  $\mu\text{l. O}_2/\text{hr.}$ ).

Table VIII. Effect of fluoroacetate on rate of acetate oxidation by acetone powders of B. leucinophagum.

Elapsed time (minutes)	$\mu\text{l. O}_2$ uptake/15 minutes		
	AC	FAC	AC (+ FAC)
15	156	29	160
30	137	13	128*
45	97	9	54
60	78	4	8

\*FAC added after 30 min.

Each flask contained 100  $\mu\text{M}$  tris, pH 7.0; 10  $\mu\text{M}$  acetate and/or 10  $\mu\text{M}$  fluoroacetate where indicated; 0.3 ml acetone powder suspension (equivalent to 500 mg wet cells) in a total volume of 2.0 ml. Center well contained a fluted filter paper and 0.1 ml 20% KOH. Shaken under air at 30°C. Data corrected for endogenous respiration (38  $\mu\text{l. O}_2/\text{hr.}$ ).

AC = acetate; FAC = fluoroacetate.

The oxidation of malonate by whole cells of B. leucinophagum was inhibited by chloramphenicol at a concentration of 200  $\mu\text{g/ml}$  (Table VI). "Normal cells," i.e. cells not previously exposed to malonate, are inhibited more than three times as much as cells grown in the presence of malonate.

Arsenite is known to inhibit the TCA cycle at the point at which  $\alpha$ -ketoglutarate is oxidatively decarboxylated to form succinyl-CoA. Table VII shows that oxygen uptake by acetone powders of B. leucinophagum in the presence of acetate, succinate and malate was also inhibited by arsenite.

Another inhibitor of the TCA cycle is fluoroacetate. This compound replaces acetate in the condensation with oxalacetate, to form fluorocitrate, which cannot be metabolized by aconitase (10). When fluoroacetate was added to a suspension of acetone-dried cells oxidizing acetate, a rapid decrease in the rate of oxidation was noted (Table VIII). Fluoroacetate itself is not significantly oxidized by acetone-dried cells of B. leucinophagum.

Further evidence for the occurrence of reactions characteristic of the TCA cycle is afforded by chromatographic analyses of reaction mixtures containing the above TCA cycle inhibitors. Large amounts of succinic acid were found to accumulate in the presence of malonate and malate, fumarate or acetate. When arsenite was included in mixtures containing malate, succinate or acetate, a carbonyl compound accumulated which, when separated as the 2,4-DNP derivative, possessed the same  $R_F$  as the derivative of authentic  $\alpha$ -ketoglutarate.

## DISCUSSION

The observations reported here, such as the rapid and abundant growth of cells on TCA cycle compounds and related compounds with a minimal lag phase from inocula grown on L-leucine as sole C and N source, the rapid oxidation of TCA cycle intermediates by whole cells, together with the fact that B. leucinophagum is obligately aerobic (5), furnish good suggestive evidence for the presence and function of a TCA cycle when B. leucinophagum is growing in the presence of L-leucine. The formation of amino acids of the aspartic-glutamic families from TCA cycle intermediates, previously suggested, would thus be in conformity with reactions known to occur in other organisms (6, 7).

The fact that malonate oxidation by whole cells takes place only after a lag period of some 40 minutes, and that this oxidation by cells not previously exposed to malonate is strongly inhibited by concentrations of chloramphenicol known to inhibit protein synthesis, suggests an adaptive process. This could involve adaptive synthesis of either a "malonate-oxidizing" enzyme system or a "malonate permease" (11). This hypothesis of adaptation is supported by the observation that malonate-grown ("adapted"?) cells are less sensitive to the action of chloramphenicol. B. leucinophagum has previously been shown (unpublished observations) to contain high concentrations of free intracellular amino acids, even when "starved" by incubation in L-leucine solutions to reduce endogenous concentrations of other growth medium amino acids.

It is also of interest that B. leucinophagum is one of the few bacterial species able to metabolize malonic acid, a compound ordinarily considered a metabolic inhibitor.

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